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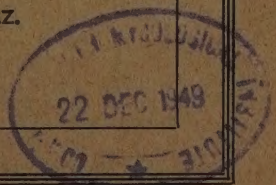

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THE NEW ZEALAND

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VOLUME 30, No. 1, 1948

(AGRICULTURAL RESEARCH SECTION)

CONTENTS

PAGE

Apple-Mosaic in New Zealand. *J. D. Atkinson and E. E. Chamberlain* ... 1

Tomato-Speck of Tomato. *W. D. Reid* ... 5

Cecidomyid Midges on Meadow Foxtail and Cocksfoot in New Zealand. *H. Jacks and W. Cottier* ... 9

Preliminary Study of the Inheritance of Grain Weight in Wheat. *S. W. Boyce* ... 13

A Note on Heterosis in a *Triticum Vulgare* Cross. *S. W. Boyce* ... 23

Cobalt, Copper and Iron in the Liver in Relation to Cobalt Deficiency Ailment. *K. J. McNaught* ... 26

Bacillus Mesentericus: An Assay Organism for Penicillin. *R. L. Nielson* ... 43

Control of Halo-Blight in Beans. *W. D. Reid* ... 45

NOTICES

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THE NEW ZEALAND

JOURNAL OF SCIENCE

AND

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Editor: D. Cairns, M.Sc. (Assistant Editor: M. O'Connor, M.Sc.) Department of Scientific and Industrial Research, Wellington

VOL. 30. SEC. A JUNE, 1948 NUMBER 1

APPLE-MOSAIC IN NEW ZEALAND

By J. D. ATKINSON and E. E. CHAMBERLAIN, Plant Diseases Division, Department of Scientific and Industrial Research

(*Received for publication, 23rd January, 1948*)

Summary

A cream and yellow mottling of apple foliage has been present in New Zealand for many years. This condition appears to be the same as that known in other countries as apple-mosaic, the causal virus of which has been listed by Smith (1937) as *Pyrus* virus 2. Infected trees have been observed in all main apple growing areas, and in several outlying orchards. The disease has been seen in 39 varieties and 3 stocks. Mosaic symptoms developed on 26 of 36 healthy apple seedlings budded with infected wood.

APPLE growers in New Zealand have known a form of yellow mottling in Jonathan foliage for many years. As this appeared to cause no injury to trees little attention was paid to it, and some growers came to regard it as a varietal characteristic. Experiments carried out over the past two years have shown that this mottling is of virus origin and is apparently identical with apple-mosaic.

SYMPTOMS

Infected apple leaves show many forms of mottling, the most common being a number of small irregular creamy or yellow spots that stand out conspicuously against the dark green of normal leaf tissue. These spots do not occur in any regular pattern, and any number from one to several hundreds may be present on a single leaf (Figs. 1 and 2). Sometimes spots are so numerous that parts of the leaf appear completely chlorotic. Where chlorosis involves a considerable area of the leaf it is common to find necrosis taking place as the season advances. More rarely bands of chlorotic tissue develop along some of the larger veins (Fig. 3). In other cases mottling may take the form of a light and dark green mosaic (Fig. 4), or of large vaguely defined patches of yellowish white tissue (Fig. 5). One or all of these symptoms may be present on a single tree, or even on a single branch. All leaves on a shoot may show symptoms, though it is more usual to find a few mottled leaves among apparently normal ones.

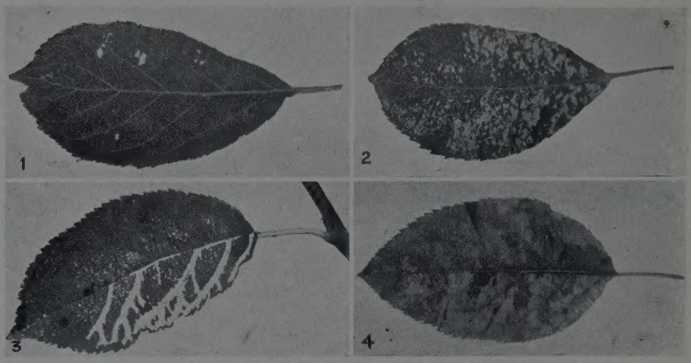

FIG. 1
Sparse mottling on Jonathan leaf infected with apple-mosaic
(× ⅔) Photo: L. H. Wright

FIG. 2
A severe case of the spotted form of apple-mosaic on a Jonathan leaf
(× ½) Photo: R. I. Hughes

FIG. 3
Bands of chlorotic tissue along veins accompanied by irregular spotting on a leaf of Golden Delicious
(× ½) Photo: L. H. Wright

FIG. 4
Mosaic pattern produced by apple-mosaic on Golden Delicious
(× ½) Photo: L. H. Wright

FIG. 5
Extensive chlorosis produced by apple-mosaic on artificially inoculated Jonathan seedlings
(Natural Size) Photo: L. H. Wright

In commercial orchards, symptoms are often pronounced on Golden Delicious and Jonathan, it being common to find over half the leaves on infected trees of these varieties showing mottling. On other

varieties, symptoms may be scattered over the tree, but it is more usual to find only a few spurs showing mottled leaves. Field observations suggest that trees showing severe symptoms are less vigorous than healthy trees. No fruit symptoms have been observed. Infection has been seen on the following apple varieties:—Apple of Commerce, Afriston, Ballarat, Beauty of Bath, Charles Ross, Cox's Orange Pippin, Delicious, Dougherty, Dunn's Favourite, Elsie Grant, Giant Jeniton, Glengyle Red, Golden Delicious, Granny Smith, Gravenstein, Jonathan, Laxton's Pearmain, Laxton's Pioneer, Laxton's Royalty, Lord Derby, Lord Lambourne, Lord Wolsely, Newton Wonder, Oratia Beauty, Parlain's Beauty, Rival, Rokewood, Russet (type unknown), Scarlet Nonpareil, Scarlet Pearmain, Scarlet Queen, Shorland Queen, St. Everard, Statesman, Sturmer Pippin, Symonds Winter, Willie Sharp, Worcester Pearmain, Yates, and the Malling Stocks Nos. IX, XII and XVI.

INCIDENCE

Apple-mosaic has been found in the five main apple growing areas, namely Auckland, Hawkes Bay, Nelson, Canterbury and Central Otago. In addition it has been found in Auckland Province at Te Hana, Te Puke, Tinopai, Tuakau and Warkworth, and in Wellington Province at Levin, and Masterton. A detailed survey was made in one Nelson orchard where every tree of 80 mature Jonathans was found to show some degree of infection. In three blocks of Dougherty and six of Granny Smith examined near Auckland, between 60 per cent. and 80 per cent. of the trees were found to be infected, but very few leaves per tree showed mottling. In three blocks of Golden Delicious all trees examined showed mosaic, the percentage of leaves affected being high. No detailed examinations have been made in other districts. In two small blocks of Jonathan each of which was raised from clonal buds, every tree showed infection; but the number of leaves showing mottling, and the severity of symptoms varied widely between trees.

TABLE I. TRANSMISSION OF APPLE-MOSAIC FROM APPLE TO APPLE BY BUDDING
Budding was carried out on 15/3/46. Records taken on 25/11/46.

Budwood Source.	Type of Seedling Inoculated.	No. of Seedlings Budded.	No. of Seedlings Infected.
Granny Smith	Granny Smith	4	2
	Jonathan	4	2
Jonathan	Granny Smith	8	5
	Jonathan	8	7
Statesman	Jonathan	1	1
	Sturmer Pippin	1	–
	Granny Smith	1	1
Golden Delicious	Jonathan	1	1
	Sturmer Pippin	1	1
	Granny Smith	1	–
Ballarat	Jonathan	1	1
	Sturmer Pippin	1	1
	Granny Smith	1	1
Elsie Grant	Jonathan	1	1
	Sturmer Pippin	1	1
	Granny Smith	1	1

38 untreated check seedlings all remained healthy.

TRANSMISSION

Seeds collected from Jonathan, Sturmer, Pippin and Granny Smith apples were grown in pots under glass during the 1945-46 season. Seedlings grew well and remained free from virus. Budwood was collected from infected trees in the field, only those shoots showing pronounced symptoms being selected. Buds from these were inserted into healthy seedlings in March, 1946, and pots then moved outside. Early in the following spring seedlings were set out in nursery rows, but stocks were not cut back. Symptoms became visible on both scions and seedling stocks within six weeks from the commencement of spring growth. Results are set out in Table I.

At budding some seedlings were smaller in diameter than the budwood. As a result only 19 of the 36 buds grew, but despite this 26 of the seedlings developed mosaic symptoms. Thirty-eight check seedlings in an adjacent row remained healthy. Symptoms which developed on seedling stocks were variable, but it was not possible to correlate type of symptom with source of inoculum or with seedling employed. In many cases several types of symptom appeared on the one seedling. Results suggest that leaf mottling in the five scion varieties was caused by the same virus.

DISCUSSION AND CONCLUSION

Vibert working in France in 1863 is reported to have observed perpetuation of variegation on apple foliage by budding, and to have noticed its spread to leaves of the stock (Bradford and Joley, 1933). At that date, infectious viruses were not recognized as such, and the earliest record of transmission of apple-mosaic as a specific disease appears to be that of Orton and Wood (1924). Since then the transmission of apple-mosaic by budding or grafting has been reported from Michigan (Bradford and Joley, 1933), California (Thomas, 1937), Bulgaria (Christoff, 1934), Nova Scotia (Hockey, 1943), South Africa (Louw, 1944), and England (Wallace *et al.*, 1944). From descriptions and figures published it is probable that all were dealing with the same virus. Christoff (1934) claimed that apple-mosaic was transmissible from apples to pears and rose haws. Thomas (1937) by budding, grafting or inarching obtained transmission from apples to *Cotoneaster harrovianna*, loquat (*Eriobotrya japonica*), *Photinia arbutifolia*, and *Sorbus pallescens*. The authors cited have between them recorded mosaic in 17 named apple varieties. Smith (1937) classified apple-mosaic as *Pyrus* virus 2.

Both in symptoms and ease of transmission by budding the New Zealand virus agrees closely with descriptions of apple-mosaic (*Pyrus* virus 2).

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TOMATO-SPECK OF TOMATO

By W. D. REID, Plant Diseases Division, Department of Scientific and Industrial Research

(*Received for publication, 31st March, 1948*)

Summary

Tomato-speck, a bacterial disease of tomato, is recorded in New Zealand. The disease on fruits is characterised by small (1 mm.) dark brown pustules surrounded by a narrow circular light halo. On leaves, lesions are usually numerous and are small (1 to 2 mm.) dark brown to black dry spots surrounded by a yellow zone. Infected plants are light in colour, stunted and unhealthy in appearance.

The morphology and the cultural characters of the causal organism are described. They agree with those given for *Pseudomonas punctulans* (Bryan) Dowson.

TOMATO-SPECK is a bacterial disease of tomato caused by *Pseudomonas punctulans* (Bryan) Dowson (Dowson, 1943). The disease was first found in New Zealand in 1944 at Hastings, where it was causing serious stunting of Tatura dwarf variety in a field crop. The crop was raised from imported seed and young plants in seedling boxes showed extensive leaf infection. The disease has not become widespread in this country but is of economic importance in the United States of America and in Canada (Conners, 1942). It has also been reported from Formosa (Okabe, 1933) and Australia (Fish, 1939).

SYMPTOMS

The name tomato-speck (Bryan, 1933) was derived from the symptoms on fruits. Lesions are very small, dark, round pustules, rarely more than 1 mm. in diameter and appear on the fruit surface as specks surrounded by a white halo (Figs. 1 and 2). They do not penetrate deeply into the flesh.

On leaves lesions are at first dark water-soaked spots. Later they become dark brown, almost black, and irregular in outline but remain small, not more than 3 mm. in diameter (Fig. 3). They are dry and rough in appearance and are surrounded by a narrow yellow halo. The spots are often crowded on the leaf surfaces giving a yellow appearance to affected foliage. Where lesions occur on leaf veins infection progresses along the veins forming narrow lesions. Natural stem lesions as described by Bryan (1933) have not been observed in New Zealand.

Infected plants in the field are often markedly stunted being less than half the height of normal plants. They are unthrifty in appearance largely because infected leaves are yellow to brown in colour and small. On fruits the many lesions are unsightly and render fruits unsuitable for marketing.

CAUSE OF DISEASE

Microscopic examination of typical lesions showed the presence of abundant bacteria and isolation on beef-peptone-agar regularly gave pure cultures of the causal organism. Numerous glasshouse tests in 1945 and 1946 involving over 50 plants showed that dwarf (Tatura and Australian Dwarf) and staked (Vetomold, Potentate and Kondine) varieties of tomatoes are susceptible to infection.

FIG. 1
Lesions on green fruit following hair injury and spray inoculation

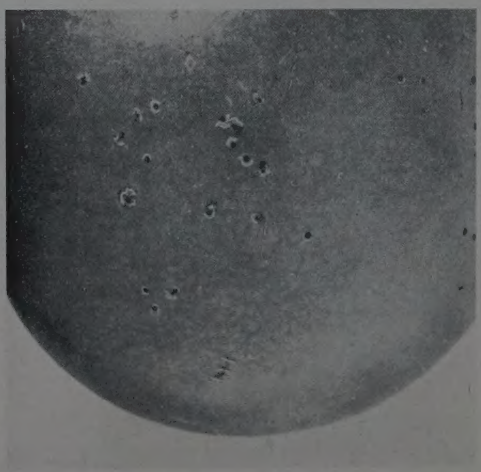

FIG. 2
Lesions on ripe fruit

Leaves, stems and fruits of plants were lightly injured by gently rubbing the surfaces with a cotton pad or flicking with a sheet of paper. The plants were then sprayed through an atomiser with a water suspension of organisms from beef-peptone-agar slopes and placed in a saturated atmosphere for 24 hours. Infection of leaves and fruits was first observed after five days as minute, black dots. These slowly increased in size to form typical lesions of the disease. Lesions were not formed on stems unless they were more severely injured by pricking or scratching. Spraying with inoculum without previous injury produced a few lesions on leaves but not on fruits or stems. Check plants injured and sprayed with water only, failed to produce obvious lesions.

FIG. 3

Leaf Lesions following brushing surface and spray inoculation

Isolation of the causal organism from lesions induced by inoculation was readily and repeatedly obtained on beef-peptone-agar. Reinoculation of tomato plants with cultures so obtained gave typical lesion formation.

The symptoms from field specimens and from glasshouse inoculations agree with those described by Bryan (1933) for bacterial-speck of tomato.

MORPHOLOGY OF CAUSAL ORGANISM

Vegetative cells after two days growth on beef-peptone-agar exist as short rods with rounded ends, singly, in pairs and as short chains. They vary in size from $0.5 \times 0.6\ \mu$ to $0.6 \times 4.8\ \mu$ for single rods and to $0.6 \times 16\ \mu$ for chains. Single rods average $0.5 \times 1.6\ \mu$. The organism is gram negative (Hucker stain) and is motile by one to three polar flagella (Leifson, Gray, Bailey and Morton stains), some rods show flagella with definite side origin.

CULTURAL CHARACTERISTICS*

Beef-peptone-agar plate colonies : Growth at 26°C. slow, circular, smooth, convex, undulate edge, greyish-white. *Beef-peptone-agar slant :* Moderate growth, convex to raised, filiform, glistening, grey, smooth, translucent, butyrous, no odour. *Gelatine colonies :* Punctiform, round to convex, undulate, smooth. *Nutrient-gelatine stab :* Slow crateriform liquefaction after 3 days. *Plain-gelatine-stab :* Slow crateriform liquefaction after 3 days. *Nutrient-broth and Tryptophane broth :* Moderate clouding in 4 days, flocculent surface growth, finely granular sediment. *Purple-milk :* Alkaline in 4 to 13 days, later acid, firm curd in 33 days, no whey. *Potato-slice :* In 9 days growth moderate, light brown, moist, smooth, potato grey. *Nitrates :* Strong nitrite production in 20 days in nitrate-broth ; weak in agar media. *Indole :* Not formed. *Hydrogen sulphide :* Not formed. *Starch :* Not hydrolysed. *Ammonia :* Not formed in 20 days in broth or agar media. *Synthetic-carbohydrate media :* No gas in any medium ; acid in arabinose, glucose, fructose, sucrose, glycerol and mannitol in 14 days ; acid in lactose and maltose in 34 days but no change in 20 days and slight change in 30 days ; no acid in 34 days in Rhamnose, raffinose, melizitose, starch, inulin, dextrin, glycogen and salicin. *M.R. test.* Positive. *V.P. test.* Negative.

The morphological, cultural and physiological characteristics of the casual organism agree with those of *Bacterium punctulans* Bryan (1933), except that the New Zealand organism has not shown ammonia production. *Bacterium punctulans* is listed by Bergey (1939) as a probable synonym of *Phytomonas tomato* (Okabe) Magrou. More recently Dowson (1943) has classified this organism as *Pseudomonas punctulans* (Bryan) Dowson. The organism under discussion produced only a slight colour change in lactose and maltose in 30 days with a definite acid reaction in 34 days. Dowson (1939) in defining the genus *Pseudomonas* states that " none produce acid in lactose, maltose and salicin " basing his conclusions on tests which did not exceed four weeks.

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* Difco media used where applicable and methods those suggested by " Manual of methods for pure culture study of bacteria " of Society of American Bacteriologists.

CECIDOMYID MIDGES ON MEADOW FOXTAIL AND COCKSFOOT IN NEW ZEALAND

By H. JACKS and W. COTTIER, Plant Diseases Division, Department of Scientific and Industrial Research

(*Received for publication, 30th April, 1948*)

Summary

1. The midge *Stenodiplosis geniculati Reut.* was reared from meadow foxtail (*Alopecurus pratensis* L.) seed-heads and *S. geniculati* Reut. var. *dactylidis* Barnes from cocksfoot (*Dactylis glomerata* L.) seed-heads collected in the provinces, Auckland, Hawke's Bay, Taranaki, Wellington, Otago and Southland. In addition the latter variety emerged from cocksfoot collected in Canterbury. No specimens of *S. geniculati* were reared from cocksfoot.

2. No specimens of *Dasyneura alopecuri* Reut. were collected during the survey and it is concluded that if this is present, it must be single-brooded as in England.

3. Colours of both sexes of *Stenodiplosis geniculati* Reut. var. *dactylidis* Barnes are described.

4. In a preliminary survey, no midges were found in seed-heads of 15 other species of Gramineae.

INTRODUCTION

DURING seasons 1937 and 1938 it was found that a Cecidomyid midge was breeding in cocksfoot (*Dactylis glomerata* L.) seed-heads in Canterbury. Specimens were sent to Dr. Barnes of Rothamsted Experimental Station, England, who stated that in his opinion the insects were a hitherto undescribed variety of *Stenodiplosis geniculati* Reut. and he described the variety under the name *dactylidis* (Barnes, 1940). The same author (Barnes, 1931) had previously stated that specimens of *S. geniculati,* reared from meadow foxtail (*Alopecurus pratensis* L.) in New Zealand were in the collection of Dr. E. P. Felt at the State Museum, Albany, N.Y., U.S.A. However, this fact was not adequately appreciated in New Zealand, it being generally considered that the Cecidomyid species on meadow foxtail here was *Dasyneura alopecuri* Reut. as reported by Cockayne (1916). In order to confirm the presence of *Stenodiplosis geniculati,* a survey on meadow foxtail was begun in 1938-39. Results of that season's survey have been reported by Barnes (1940), who identified all specimens reared as *S. geniculati.* He also mentioned he thought it likely that the species was present in New Zealand in 1916 and that possibly Cockayne (loc. cit.) was unknowingly dealing with the effects of both *S. geniculati* and *Dasyneura alopecuri.* The survey on meadow foxtail was continued in 1939-40 and extended in that season to include cocksfoot in order that the incidence of the variety *dactylidis* in the Dominion could be determined; a preliminary search for midges on other grasses was also carried out. It is the purpose of the present paper to record and discuss results obtained in 1939-40.

MEADOW FOXTAIL AND COCKSFOOT, 1939-40

Material and Methods

During this season, through the courtesy of the Fields Division of the Department of Agriculture, seed-heads of these grasses were sent to Auckland from many parts of New Zealand. Meadow foxtail was collected from November to February and cocksfoot from December to March. Altogether a total of 48 samples of meadow foxtail and 52 of cocksfoot were received. The localities in which these were taken were:

Province.	Meadow Foxtail.	Cocksfoot.
Auckland	Auckland City, Dargaville, Tauranga, Te Kuiti, Warkworth, Whakatane.	Auckland City, Warkworth, Whakatane.
Hawke's Bay	Dannevirke, Hastings, Takapuna, Waipukurau.	Dannevirke, Hastings.
Taranaki	New Plymouth, Stratford.	New Plymouth.
Wellington	Palmerston North, Wanganui.	Palmerston North, Wanganui.
Canterbury		Ashburton, Christchurch.
Otago	Dunedin, Ranfurly.	Balclutha.
Southland	Invercargill, Otatara.	Invercargill.

Fifty seed-heads were selected at random from each sample and each lot of 50 was placed in a separate quart glass preserving jar, the mouth of which was covered with muslin. These were kept under observation in the laboratory for 60 days. Emergence began a few days after receipt of samples and from then onwards midges were counted and removed daily.

TABLE I. MEAN NUMBERS OF MIDGES EMERGED PER SEED-HEAD OF MEADOW FOXTAIL AND COCKSFOOT

Grass and Provinces.	Nov.	Dec.	Jan.	Feb.	Mar.	Totals.
Meadow Foxtail						
Auckland	65.6* (7)	14.0 (3)	1.0 (3)	—	—	80.6 (13)
Hawke's Bay	16.8 (4)	12.0 (5)	—	—	—	28.8 (9)
Taranaki	0.0 (1)	3.1 (1)	—	—		3.1 (2)
Wellington	21.5 (4)	0.5 (1)	0.3 (2)	—	—	22.3 (7)
Otago	0.0 (3)	9.8 (4)	0.8 (4)	—	—	10.6 (11)
Southland	0.1 (2)	1.3 (1)	0.2 (2)	0.5 (1)	—	2.1 (6)
Cocksfoot						
Auckland	—	47.1 (2)	36.9 (11)	114.8 (9)	101.1 (2)	299.9 (24)
Hawke's Bay	—	71.3 (5)	0.0 (1)	—	—	71.3 (6)
Taranaki	—	63.7 (2)	0.7 (1)	0.0 (2)	—	64.4 (5)
Wellington	—	29.9 (2)	4.7 (3)	0.4	—	35.0 (5)
Canterbury	—	0.7 (2)	2.8 (5)	0.0 (2)	—	4.5 (9)
Otago	—	1.0 (1)	—	—	—	1.0 (1)
Southland	—	0.0 (1)	—	1.7 (1)	—	1.7 (2)

* Figures in brackets show numbers of samples received.

RESULTS

These are summarized in Table I where they are expressed as mean numbers of midges that emerged per seed-head. Localities of collection have been grouped into provinces, the figures in brackets showing the number of samples received per month from each province. A dash (—) shows that no samples from the province in question were received during the month.

DISCUSSION

It is clear that midges are distributed throughout the country, as some emerged from both meadow foxtail and cocksfoot in every locality sampled.

In the case of meadow foxtail, greatest numbers of midges per seed-head emerged from material collected in the Auckland Province. In the North Island, excluding the two Taranaki samples, midges emerged from this grass more abundantly during November than in any other month and they became progressively fewer up to February. Suitable seed-heads of meadow foxtail were becoming difficult to secure in these localities by mid-January because of dropping of seed and were unprocurable in February. In the South Island, indications were that maximum emergence was later than in the North, as this occurred in Otago and Southland in December. Total mean emergence per seed-head was also less in these provinces than in the north.

In the case of cocksfoot, emergence over the period December to March was also greatest in Auckland. In this province, however, many of the collections were made from small stands of cocksfoot about the city and may not indicate the true position in farming areas. Infestation in the North Island was considerable during December and, with the exception of Auckland, became less in the succeeding months. As with the meadow foxtail total, mean emergence of midges per seed-head was less in the South Island than in the North Island provinces.

In view of the statements of Cockayne (loc. cit.) and Miller (1918) that the midge on meadow foxtail had a serious effect on seed production, sometimes destroying as much as 70 per cent. of the crop, it would seem probable from the figures in Table I that the cocksfoot midge is equally destructive to the seed of its host.

IDENTITY OF MIDGES

All collections of midges from meadow foxtail and cocksfoot were carefully examined for identification of species. In view of the statement of Barnes (1940 and 1946) that *Stenodiplosis geniculati* occurs on cocksfoot in England and Ireland, and that he had bred the species through successfully on cocksfoot without any alteration in the characters of typical meadow foxtail specimens, it was thought that specimens of *S. geniculati* might be found among the midges from cocksfoot. Examination, however, failed to disclose this, all specimens from meadow foxtail being *S. geniculati* and all from cocksfoot *S. geniculati* var. *dactylidis*.

Dasyneura alopecuri Reut.

When this survey was originally planned it was thought that some evidence of the occurrence of *Dasyneura alopecuri* might be obtained. However, perusal of the life-cycle of this species in England shows that there it is single-brooded, with a possibility of a second brood in

very favourable seasons (Barnes, 1930). The fact that no specimens of *D. alopecuri* were reared from meadow foxtail during the New Zealand survey does not mean that the species is absent from the Dominion, but it does show that if it is present as stated by Cockayne (loc. cit.) and Miller (loc. cit.), it must be single-brooded as in England.

Colours of Stenodiplosis geniculati var. dactylidis

As no previous record of the colours of this variety in life has been published, the following notes concerning them have been made.

Adult female.—Head and thorax very dusky to black. Antennae and eyes black. Halteres dusky, tinged with red. Wing insertions reddish-yellow. Wings grey, veins dusky. Legs dusky, tinged with light yellow. Abdomen brick red ornamented with dusky areas; apical two segments black.

Adult male.—Head and thorax dusky to black. Eyes black. Antennae dusky tinged with yellow. Abdomen yellow, dusky at apex. Other characters as in female.

It is interesting to note that the abdomen in the adult female is red. In his key for separation of midges attacking cocksfoot, Barnes (1940) gave the colour of the abdomen in adults of *S. geniculati* from that grass as dull honey-yellow, so that the variety *dactylidis* is further distinguished in the adult female by colour. This red colour fades rapidly after death.

OTHER GRASSES, 1939-40

In a preliminary survey for the presence of midges, seed-heads of the following grasses were collected in the neighbourhood of Owairaka, Auckland. Perennial ryegrass (*Lolium perenne* L.), Italian ryegrass (*Lolium multiflorum* Lam.), prairie grass (*Bromus catharticus* Vahl.), sweet vernal (*Anthoxanthum odoratum* L.), Yorkshire fog (*Holcus lanatus* L.), *Paspalum dilatatum* Poir., tall oat grass (*Arrhenatherum elatius* (L.), Mert. et Koch), oats (*Avena sativa* L.), wheat (*Triticum aestivum* L.), tall fescue (*Festuca arundinacea* Schreb.), hair grass (*Vulpia dertonensis* (All.) Volk), red-top (*Agrostis alba* L.), Bermuda grass (*Cynodon dactylon* (L.) Pers.), Kentucky blue-grass (*Poa pratensis* L.) and Chewing's fescue (*Festuca rubra* L. var. *fallax* Hack.). The seed-heads were kept in the laboratory under the same conditions as were those of meadow foxtail and cocksfoot but no midges emerged from any of them.

ACKNOWLEDGMENT

Thanks are offered to those officers of the Fields Division, Department of Agriculture, who assisted by sending in grass samples.

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A PRELIMINARY STUDY OF THE INHERITANCE OF GRAIN WEIGHT IN WHEAT

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(*Received for publication, 1st September, 1947*)

Summary

Inheritance of grain weight has been studied in three crosses of *Triticum durum* and one of *Triticum vulgare*. In one case there is evidence of segregation of a single recessive gene for high grain weight. In the remaining crosses, high grain weight is either completely or partially dominant, differences being determined by two or three major genes. Secondary effects of genes controlling other components of yield probably also contribute to differences in grain weight.

Introduction

The following study of inheritance of grain weight in wheat was undertaken in an attempt to measure single gene effects and to estimate numbers of genes segregating in different crosses.

It was first necessary to study the nature of dominance deviations.† At one time it was assumed that dominance did not occur in quantitative characters but it has now been demonstrated effectively in numerous cases, though it is not always present. Sax (1923) demonstrated absence of dominance in *Phaseolus* crosses for seed size and Wexelsen (1933) obtained evidence of dominance in opposite directions in barley. We still see occasional references to the general absence of dominance in quantitative characters, e.g., Richey (1945) in developing a method for augmenting vigour in inbred lines of corn, makes the absence of dominance in quantitative characters one of his basic assumptions; he considers that genes for vigour which display dominance are distinct from genes controlling quantitative characters in general, which, according to his theory do not display dominance. Since vigour is measured quantitatively it is very doubtful whether such a distinction between the two types of gene can be drawn. The present studies being made on yield in wheat may lead to further knowledge of such dominance relationships.

Estimates of the numbers of genes controlling kernel weight in wheat have already been published by Jasnowski (1934, 1935), who obtained evidence of three gene differences between certain lines. On the other hand, Worzella (1942) states that kernal weight is multigenic. In the following experiments it appears that one to three major genes are segregating, but the possibility of secondary minor gene differences is not disproved.

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† The expression "dominance deviations" refers in this paper to deviations of the phenotypic values of heterozygotes from the arithmetic mean of the two corresponding homozygotes when gene differences are additive, and from the geometric mean when gene differences are geometric.

MATERIAL AND METHODS

Four crosses were studied. The first three were crosses between varieties of the 28 chromosome wheat, *T. durum*. Of these 150 plants of the parent lines and 400 plants of the F_2 generation were grown in one season. A sample of 100 grains from each plant was weighed, the mean weight of 1 grain being calculated in units of .001 g. The fourth cross was made between two varieties of *T. vulgare*, which has 42 chromosomes; of this were grown in one year, about 1,000 plants of each parent, 200 F_1 plants, 400 F_2 plants and over 5,000 F_3 plants. In this case all grains of each plant were counted and weighed, the mean weight of one grain being again estimated in units of .001 g.

ANALYSIS OF RESULTS

The distribution of grain weight was first plotted for a few parent lines using a class interval of 1 unit. From the standard deviation of these preliminary distributions, a corrected class interval was obtained (Sinnott and Dunn, 1932).

T. durum :

Of line E252 the standard deviation was 5.43, approximately $\frac{1}{3}$ of which, or 2 units, was chosen as class interval. Since it was apparent that the genetic differences were fairly simple this value was later doubled to 4 units for greater ease in handling data.

T. vulgare :

In 1942 four pure lines were given a preliminary analysis :

S 1884	standard deviation	5.77	units
S 1612	,, ,,	4.58	,,
S 1556	,, ,,	3.10	,,
S 357	,, ,,	3.20	,,
Average of standard deviations		4.16	,,

A class interval of 1.5 units was therefore chosen for use in 1942, weight having been recorded to three figures which allowed this subdivision of classes. In the following year, owing to adverse field conditions, the parents were more variable, the standard deviation of S 357 being 5.92, and that of S 1556, 3.99. The class interval was therefore increased to 2 units, which was approximately $\frac{1}{2}\frac{1}{0}$ of the range of each of the above lines.

It should be stressed here that the choice of class interval is in any case rather arbitrary, and in some cases may have to exceed the statistically ideal standard of $\frac{1}{3}$ to $\frac{1}{4}$ of the standard deviation, considerations such as ease of handling data determining a larger class interval.

The preliminary investigations of pure lines showed firstly, a general difference between the range of the two species, (Fig. 1), indicating one major type of genetic difference which we shall discuss later. Secondly, within each species there are again distinct differences in both means and variances though some lines are almost identical. Finally, within pure lines were found large seasonal differences ; Fig. 2 illustrates the difference between four plots of one line, S 357, grown in two different positions in each of two years. The major difference here is that between years and it illustrates the importance of eliminating seasonal differences from quantitative studies wherever possible.

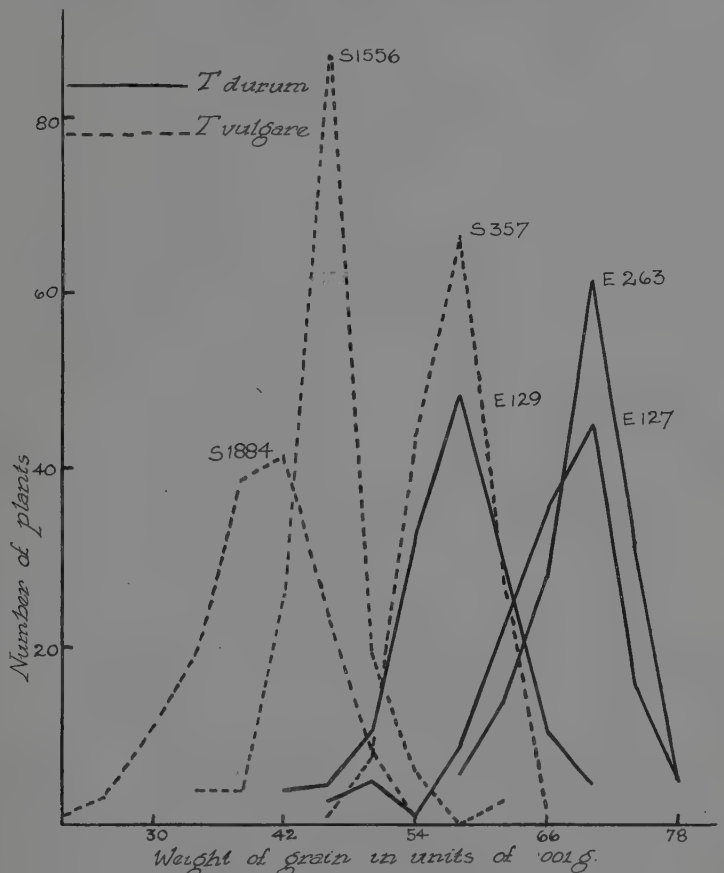

FIG. 1.—Plantwise distributions of grainweight of three *T. vulgare* and three *T. durum* wheats.

ANALYSIS OF SEGREGATION

T. durum crosses.

The grain weights of P_1, P_2 and F_2 are shown in Table I, the lower weight parent being P_1 in each case.

TABLE I. *T. durum* CROSSES: MEANS OF P_1, P_2 AND F_2 FOR THREE CROSSES

Cross.	Grain Weight.		
	P_1	P_2	F_2
1	57.6 ± .47	66.2 ± .52	61.3 ± .42
2	58.6 ± .60	67.6 ± .46	64.9 ± .42
3	62.1 ± .35	69.4 ± .54	67.6 ± .35

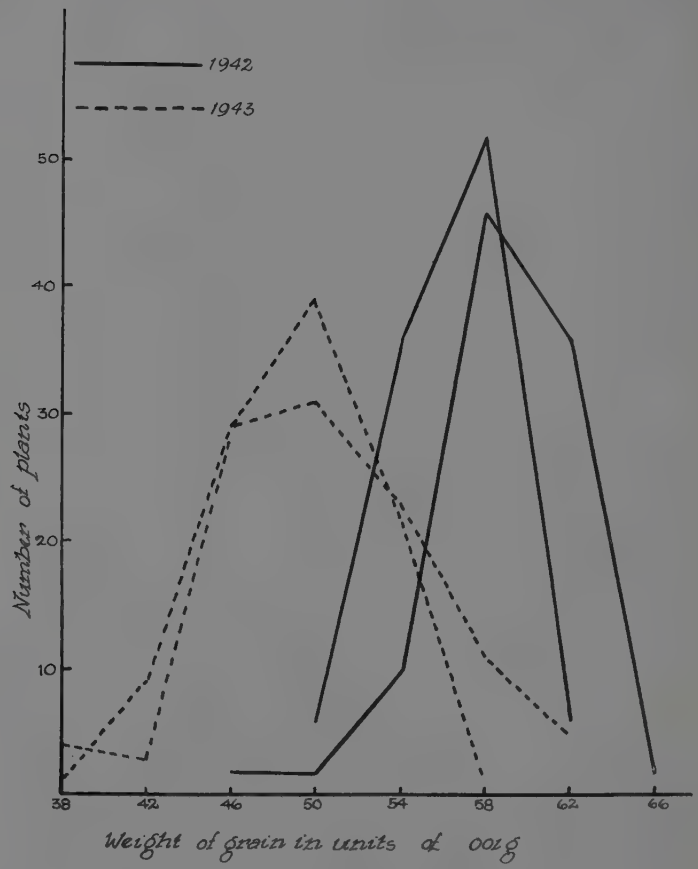

FIG. 2.—Comparison of 1942 and 1943 grain weight distributions, within and between plots.

Cross 1. E129 (Valencia) × E217 (Portugal).

The F_2 mean approaches P_1 and the first assumption is made that a single gene, dominant for low grain weight, is segregating.

Assuming that a single dominant gene is segregating one would have an F_2 composed of P_1 and P_2 types in the proportion of $3P_1 : 1P_2$. Since there are 397 individuals in the F_2 population we should expect $297.75P_1 : 99.25P_2$. The $297.75P_1$ plants should be distributed about the mean (57.6) in much the same manner as in the pure line apart from a certain additional error variance which is to be expected from the mixing of P_1 and P_2 types in the F_2. Since there were 149 plants in the pure line, we may construct a theoretical distribution by multiplying each class of the actual distribution by $\frac{297.75.}{149}$. Similarly the theoretical distribution of P_2 types in the F_2 will be obtained from the actual P_2 multiplied by $\frac{99.25.}{151}$

TABLE II. COMPARISON OF ACTUAL AND THEORETICAL F_2 DISTRIBUTIONS, ASSUMING A 3 : 1 SEGREGATION (CLASS 46 INCLUDES ALL VALUES BELOW AND UP TO 46 ; CLASS 74 INCLUDES ALL VALUES FROM 74 UPWARDS)

Class value. Distribution of :	46	50	54	58	62	66	70	74	T
Actual $P_1 \times \frac{297.75}{149}$	17.98	21.98	65.94	97.92	61.95	21.98	9.99		297.74
Actual $P_2 \times \frac{99.25}{151}$	1.97	3.29	.66	5.92	19.72	23.66	30.24	13.81	99.27
Theoretical F_2	19.95	25.27	66.60	103.84	81.67	45.64	40.23	13.81	397.01
Actual F_2	20	19	45	94	86	56	44	33	397
Difference	.05	6.27	21.60	9.84	4.33	10.36	3.77	19.19	.01
x^2	.00	1.56	7.01	.93	.23	2.35	.35	26.67	39.10
									$P<.01$

The theoretical F_2, obtained by assuming these two distributions, is shown in the third line of Table II. The next line of this table gives the actual F_2 distribution and below this is shown the x^2 value for each class. The total x^2 value is 39.10 which indicates more than random difference between the two populations. The source of this deviation is shown quite well in Fig. 3a, where the actual F_2 curve lies above the theoretical curve throughout the whole range. This can be corrected by postulating partial, but almost complete dominance so that instead of $3P_1 : 1P_2$ we have $1P_1 : 2$" F_1" $: 1P_2$. Now there is no F_1 curve for comparison but a hypothetical curve has been constructed by increasing the mean of P_1 to 59.6 (Table III). The result is shown in Fig. 3b, x_2 is now not significant, and the two curves agree very closely.

TABLE III. COMPARISON OF ACTUAL AND THEORETICAL F_2 DISTRIBUTIONS, ASSUMING A 1 : 2 : 1 SEGREGATION, THE F_1 SHOWING PARTIAL DOMINANCE (CLASS 46 INCLUDES ALL VALUES BELOW AND UP TO 46 ; CLASS 74 INCLUDES ALL VALUES FROM 74 UPWARDS)

Class value. Distribution of :	46	50	54	58	62	66	70	74	T
Actual $P_1 \times \frac{99.25}{149}$	5.99	7.33	21.98	32.64	20.65	7.33	3.33		99.25
Actual $P_1 \times \frac{198.50^*}{149}$	8.65	10.66	29.31	54.62	53.29	27.98	10.66	3.33	198.50
Actual $P_2 \times \frac{99.27}{151}$	1.97	3.29	.66	5.92	19.72	23.66	30.24	13.81	99.27
Theoretical F_2	16.61	21.28	51.95	93.18	93.66	58.97	44.23	17.14	397.02
Actual F_2	20	19	45	94	86	56	44	33	397
Difference	3.39	2.28	6.95	.82	7.66	2.97	.23	15.86	.02
x^2	.69	.24	.93	.01	.63	.15	.00	14.68	17.33
									$P=.015$

* Whole P_1 distribution has been moved up half a class interval thus increasing the mean of the theoretical F_1 to 59.6 as compared with 57.5 in the previous case.

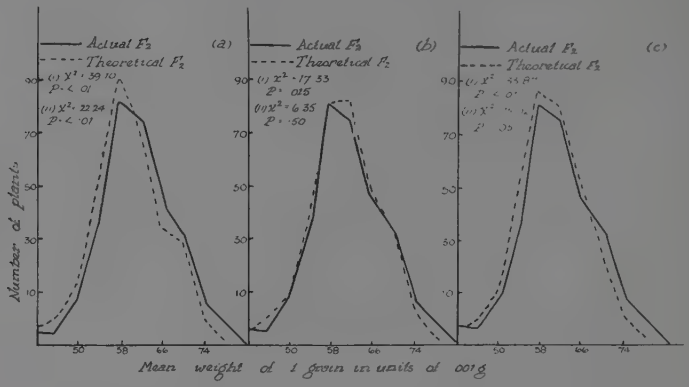

FIG. 3.—Comparison of theoretical and actual F_2 distributions, *T. durum* cross 1.

(a) $3P_1 : 1P_2$. (b) $1P_1 : 2F_1 : 1P_2$.

(c) $9P_1$: 6 intermediate : $1P_2$.

(i) x^2 calculated over whole range.

(ii) x^2 calculated from 46 to 74 inclusive.

It will be asked, would not equally close agreement be obtained by constructing theoretical curves for two or more genes? For two genes, assuming that the parent types are the extremes we have $9P_1$: 6 Intermediate : $1P_2$ (Fig. 3c). The intermediate is necessarily quite hypothetical and has been obtained by moving the P_1 distribution up to an intermediate mean value. Agreement as shown in Fig. 5 is not as good as for the simpler assumption, which can be accepted in so far as it is not disproved.

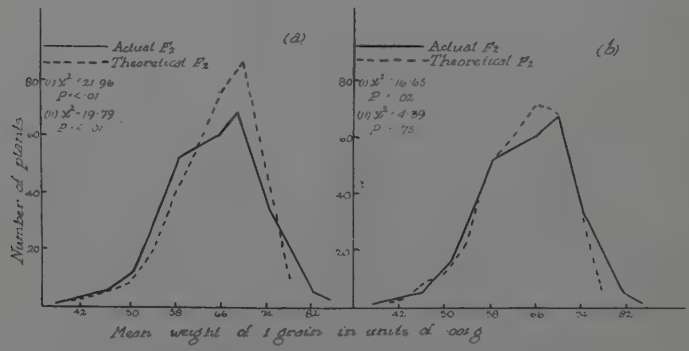

FIG. 4.—Comparison of theoretical and actual F_2 distributions, *T. durum* cross 2.

(a) $1P_1 : 3P_2$. (b) $7P_1 : 9P_2$.

(i) x^2 calculated over whole range.

(ii) x^2 calculated from 46 to 74 inclusive.

Cross 2. E156 (Portugal) × E252 (Morocco).

Since the F_2 mean approaches P_2 the first assumption is made that a single gene dominant for large grain weight is segregating. Fig. 4a shows quite clearly that a 1 : 3 segregation is not a satisfactory assumption x_2 having a highly significant value. The error appears to be due to exaggeration of the frequency of P_2. An assumption of an intermediate F_1 value does not appear to be reasonable, since the actual F_2 tends to be bimodal, indicating a possibility that there are two classes. Such a curve would arise if two complementary genes were segregating, giving a ratio of $7P_1 : 9P_2$. This distribution has therefore been tested and gives good agreement between theoretical and actual values (Fig. 4b).

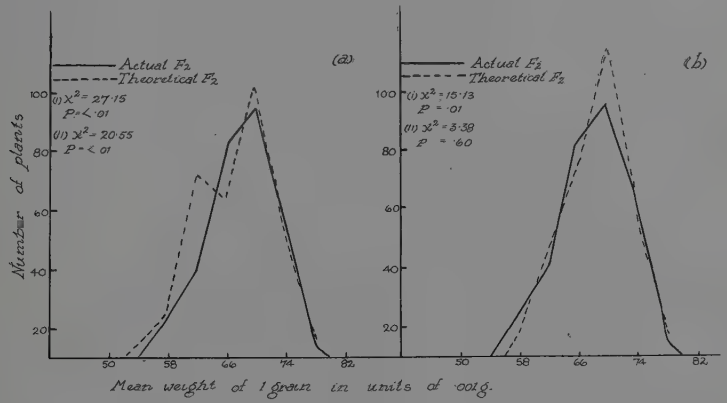

FIG. 5.—Comparison of theoretical and actual F_2 distributions, *T. durum* cross 3.
(a) $1P_1 : 3P_2$. (b) $1P_1 : 6$"F_1" $: 9P_2$.
(i) x^2 calculated over whole range.
(ii) x^2 calculated from 58 to 78 inclusive.

Cross 3. E178 (Portugal) × E263 (Morocco).

This again gives poor agreement for a 1 : 3 segregation (Fig. 5a), and as it does not show an obvious bimodal distribution, a 1 : 6 : 9 segregation for two genes has been tested (Fig. 5b), with quite good results. Various assumptions were tested for this cross, none being more satisfactory than the 1 : 6 : 9 segregation which is tentatively accepted.

To summarize the 28 chromosome *T. durum* crosses then, we find that in one cross low grain weight was partially dominant, the parents differing by one gene.

In the second cross the parents differed by two complementary genes dominant for high grain weight and in the third, high grain weight is again dominant, the number of genes being indefinite, but possibly equal to two.

T. vulgare cross. S357 (Crete) × S1556 (India).

T. vulgare wheats, as was shown in Fig. 1, tend to have lower grain weights than varieties of *T. durum* even though, according to mathematical expectations the larger number of chromosomes might be

expected to carry a greater number of genes. This specific decrease in size is probably due to a complete alteration in the balance and interaction between chromosomes and it would in fact be a waste of time to attempt to interpret such a size difference in terms of number of genes. Within the species however, it is reasonable to attempt an analysis of the number of genes segregating.

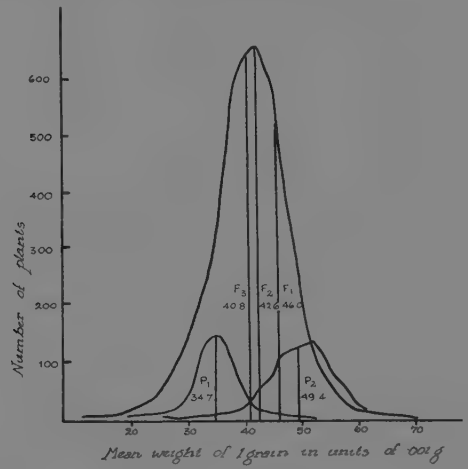

FIG. 6.—*T. vulgare* cross. Plantwise distributions of P_1, P_2, F_3 with means of P_1, P_2, F_1, F_2, F_3 shown by vertical lines.

Fig. 6 shows the actual frequency distribution and means of P_1, P_2 and the F_3 generation, with the F_1 and F_2 means marked.

From this diagram it is clear that the F_1 (46) approaches the higher parent (49.4), and this, in conjunction with the lowering of the F_2 mean to 42.6 and the F_3 to 40.8 indicates that the balance of dominance is for high grain weight. The dominance is not complete however, and the intermediate nature of the F_1 shows that either all genes segregating are only partially dominant, or that some genes are dominant for high grain weight and a fewer number are either dominant for low grain weight or are non-dominant. Both situations may of course occur simultaneously.

Considering the F_2 and F_3 distributions the relative frequency of the plants is too low for the hybrid distribution to be explained on a single gene basis, but a two gene segregation appears to be fairly reasonable. To compare the two assumptions of partial dominance and incomplete dominance, the following theoretical distributions have been constructed (Fig. 7).

1. Two genes segregating—both partially dominant, the theoretical F_1 coinciding with the actual F_1.

2. Three genes segregating two completely dominant for high grain weight, and one completely dominant for low grain weight the theoretical F_1 coinciding with the actual F_1.

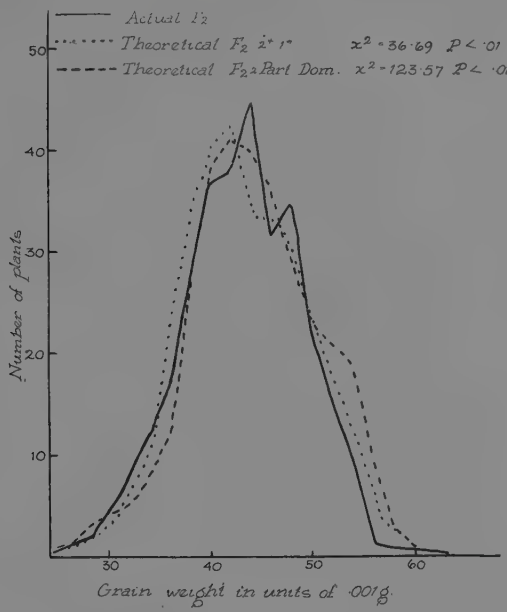
Actual F2
Theoretical F2 2+1
x² = 36·69 P < ·01
Theoretical F2 1 Part Dom.
x² = 123·57 P < ·01
Number of plants
Grain weight in units of ·001g.

FIG. 7.—*T. vulgare* cross. Comparison of actual F_2 distribution with two theoretical F_2 distributions.

Both these assumptions give quite good agreement in the F_2, the first being rather more satisfactory than the second. In the F_3 however, agreement is not very good (Fig. 8a), although it is very much better than that expected for a five gene segregation (Fig. 8b).

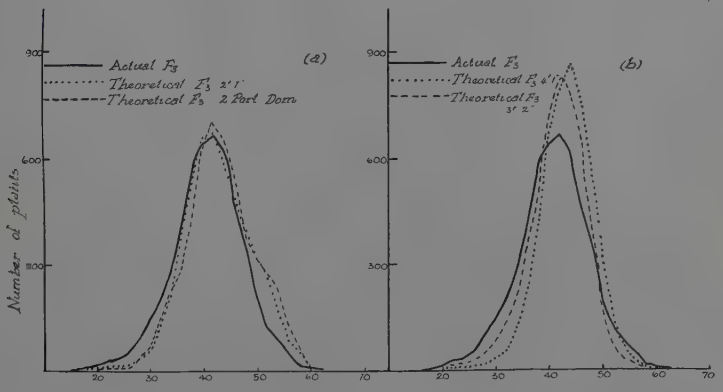

FIG. 8.—*T. vulgare* cross. Comparison of actual F_3 distribution with theoretical distributions for (a) two or three gene segregations, (b) five gene segregations.

Increasing numbers of genes were tested for each of the two main assumptions, but they gave increasingly poor comparisons with the actual data. Fig. 8b for example shows the expected F_3 distribution for five genes, four dominant + and one dominant −, the F_1 coinciding with the actual F_1, and with geometrically increasing class intervals ; and also for five genes three dominant + and two dominant −.

It should be emphasized here that the apparent one, two or three gene segregations may result from the segregation of tightly linked gene complexes, and a further programme of repeated crossing might result in the breakdown of these complexes (Mather, 1943).

DISCUSSION

The experiments described in this paper were of an exploratory nature and definite conclusions cannot be drawn. There are clear indications, from the nature of the F_2 distributions, that a few major genes are segregating, determining the general shape of the F_2 curves. However, agreement of theoretical and actual curves is not perfect, and the discrepancies may be due to the segregation of one to many minor genes. In addition, in each cross discussed above, where the segregation of more than one gene was postulated, equal effects were assumed for all the genes. This gave sufficiently good agreement for the general shape of the F_2 and F_3 curves ; but, apart from the polyploid nature of *Triticum*, there is no evidence in support of the assumption of equal gene differences (Pease, 1940). Since no more than three genes have been postulated, it is possible that these may occur on chromosomes of common origin, but even so, they may no longer exhibit equal effects. Only a long term selection programme can decide the relative importance of the size differences due to major and minor genes in this material. Minor fluctuations are likely, of course, to result from secondary effects of major gene differences affecting the development of other components of yield ; it is hoped that a more detailed study, now under way, of all yield components, will reveal the extent and nature of such apparent " minor " gene differences.

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A NOTE ON HETEROSIS IN A *TRITICUM VULGARE* CROSS

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(*Received for publication, 1st September, 1947*)

L. Powers (1944) suggests an expansion of Jones' theory for the explanation of heterosis. In studying certain maize crosses, Powers separates yield into its components and shows that heterosis in total yield depends on the multiplication together of these components which, individually may not display any degree of heterosis. A similar situation which is at present being studied in wheat is briefly reported here.

Total yield per plant (Y) in wheat is determined by the product of number of ears per plant (e), number of grains per ear (n) and mean weight of one grain (g). In one cross it was found that P_1 carried genes for high n, and P_2 genes for high e and high g, the F_1 combining all these desirable characters to give positive heterosis in Y. From Table I

TABLE I. MEAN YIELD AND ITS COMPONENTS IN P_1, P_2 AND HYBRID GENERATIONS

Generation.	Yield Component.				Yield.
	e	n	g(.001g.)	ng(g.)	Y(g.)
P_1	4.80 ± .08	26.6 ± .27	34.7 ± .16	.923	3.86 ± .10
P_2	5.47 ± .08	18.7 ± .16	49.4 ± .20	.924	5.10 ± .10
F_1	6.16 ± .17	25.0 ± .44	46.0 ± .44	1.150	6.77 ± .27
F_2	5.21 ± .11	21.3 ± .40	42.6 ± .39		4.55 ± .15
F_3	4.88 ± .03	20.5 ± .09	40.8 ± .10		3.91 ± .03

it can be seen that e is the only component of yield which displays heterosis, high n and high g being only partially dominant. Since, however, the F_1 value for e is only 12.6 per cent. higher than P_2, while the F_1 for Y is 32.7 per cent. higher than P_2, it is evident that the remaining 20.1 per cent. increase in yield is due to ng. That is, the multiplication of e by two partially dominant components of yield has greatly increased the hybrid vigour of the F_1. Figs. 1, 2, 3 and 4 illustrate the heterosis of e and Y and the partial dominance of n and g. In each case the drop in mean from F_1 to F_2 and to F_3 is typical of genes dominant for high yield. The extremely skew distribution of the F_3 for Y (Fig. 4) is due to the positive correlation of yield components and must not be confused with skewness due to dominance of genes for low yield.

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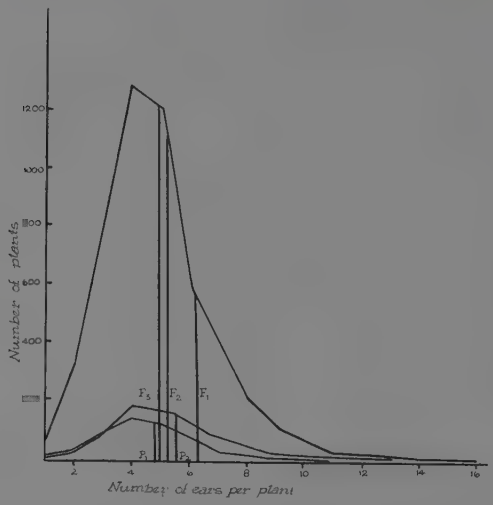

FIG. 1.—Frequency distributions of P_1, P_2, F_3 with means of P_1, P_2, F_1, F_2, F_3 for number of ears per plant.

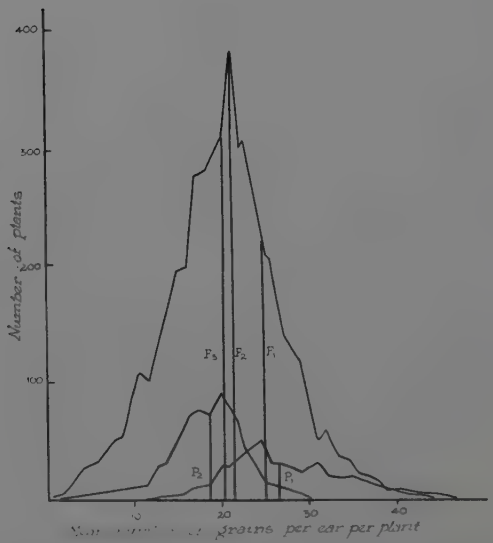

FIG. 2.—Frequency distributions of P_1, P_2, F_3 with means of P_1, P_2, F_1, F_2, F_3 for mean number of grains per ear per plant.

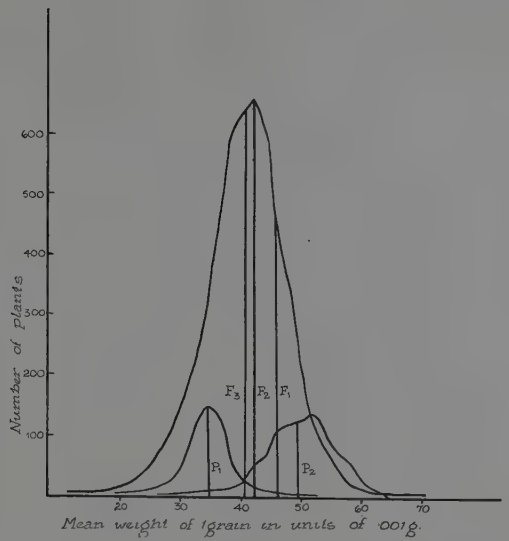

FIG. 3.—Frequency distributions of P_1, P_2, F_3 with means of P_1, P_2, F_1, F_2, F_3 for mean weight of one grain, per plant.

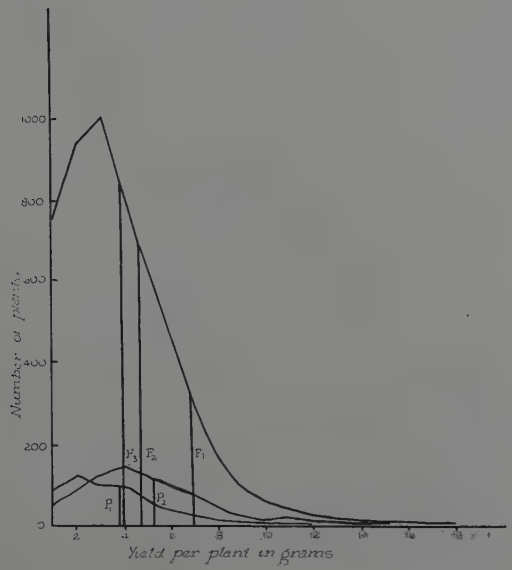

FIG. 4.—Frequency distributions of P_1, P_2, F_3 with means of P_1, P_2, F_1, F_2, F_3 for yield per plant.

COBALT, COPPER AND IRON IN THE LIVER IN RELATION TO COBALT DEFICIENCY AILMENT.

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Summary

Good correlation has been shown between both cobalt concentration and total cobalt in the liver and incidence of bush-sickness in the North Island. Evidence is submitted that the correlation is with cobalt deficiency ailment and not with unthriftiness. Some low figures have been found for healthy animals from marginal deficiency areas.

The copper content of the liver in bush-sickness is much higher than in enzootic marasmus and coast disease. No gross accumulation of iron in the liver is observed in bush-sickness.

The reference data reported have been successfully used for several years for chemical diagnosis of cobalt deficiency. Reference criteria for the interpretation of the analytical results are given.

INTRODUCTION

EARLY analyses of livers from healthy and bush-sick North Island sheep (1, 2, 3) indicated some correlation between cobalt content and incidence of bush-sickness. As the information from this and other related work (4) was not sufficient for diagnostic purposes, a more extensive survey was undertaken commencing early in 1938. War delayed the completion of this work, the combined results of which are presented in this paper. In the intervening period further results for cobalt in livers of sheep have been published (5, 6, 7, 8, 9, 10), and these are in general agreement with the values here reported.

Owing to the fact that "coast disease" in South Australia was shown to be a combined cobalt and copper deficiency ailment (11, 12), while both this disease and enzootic marasmus in Western Australia were shown to be accompanied by abnormal accumulation of iron in the liver (12, 13), copper and iron were also determined on most samples. A detailed investigation of the copper status of sheep and cattle in New Zealand has since been made by Cunningham (14).

MATERIALS ANALYSED

Liver samples analysed were from the following groups:

(a) Healthy animals from districts not affected by typical bush-sickness. Through the co-operation of the District Superintendents of the Live-stock Division of the Department of Agriculture, and the local meat and stock inspectors, samples from animals of known history were obtained.

(b) Healthy treated animals from typical "bush-sick" areas (mostly from Mamaku).

(c) Known "bush-sick" animals, that is, animals which had been grazing on a known deficiency area and which showed symptoms consistent with cobalt deficiency.

(d) Animals suffering from ailments other than cobalt deficiency (i) facial eczema (ii) enzootic icterus or toxaemic jaundice (iii) fed toxic amounts of copper (iv) copper deficiency (v) fed excessive doses of cobalt.

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Though the most susceptible animals are lambs of 4 to 9 months and calves or yearlings of 6 to 18 months, older animals, especially among sheep, will succumb to bush-sickness if grazed long enough on severely deficient pastures without cobalt supplements. Lambs younger than 4 months and calves younger than 6 months do not usually show gross symptoms of bush-sickness, but the progeny of bush-sick ewes and cows seldom thrive, even at such early ages (9). As livers from animals of widely varying ages are frequently submitted for determination of cobalt status it was considered desirable to have reference data, where possible, for animals of all ages from foetal stage to maturity.

CHEMICAL METHODS

Removal of Organic Matter

For cobalt determinations in the later work the wet digestion technique (3) was replaced by the following nitric acid ashing procedure, because of the difficulty experienced in fuming off the residual sulphuric acid conveniently and without losses by creep.

A 10g. dry weight of sample, or about 30g. wet tissue, is warmed on the hot plate in a fume cupboard in a 4 in. silica basin with about 25 ml. 50 per cent. nitric acid (equal volumes concentrated nitric acid and water), till frothing commences. The basin is removed from the hot plate and 5 ml. amyl alcohol added to suppress the frothing. Part of the alcohol reacts with the nitric acid and part boils off. When the reaction ceases, heating is continued till charring. The charred residue is ashed in the fume cupboard at 550 to 600° c. for about 30 minutes. The basin is cooled, about 15 ml. 50 per cent. nitric acid added, and, with a clock-glass on the basin to produce reflux, heating is continued till all the acid has been driven off. The residue is again ashed for 5 minutes, preferably at a lower temperature, about 500° c., to avoid damage to the basin by fusion of the ash. The nitric acid treatment followed by ashing is repeated till a residue completely free from carbon is obtained.

The above method is suitable for approximate determinations of copper on the same sample but appears to be unsuitable for the simultaneous determination of iron, for which a separate digest is necessary. The wet digestion procedure (3) has the advantage that it allows of reasonably accurate copper and iron determinations on the same digests.

Preparation of Extracts for Cobalt Determination

The subsequent treatment of the ash is similar to that previously outlined (3) except that the final evaporation to dryness is carried out at a higher temperature, for example on the sandbath, to ensure that no traces of aqua regia remain. If a permanganate-like colour develops, re-evaporation to dryness with hydrochloric acid is used.

Method of Estimation

Cobalt

All analyses prior to December, 1936, including the results reported by Josland (15, 16), were obtained by a modification of the method of Stare and Elvehjem (17) in which neutralization of the test solution is carried out with caustic potash in the presence of the nitroso-R-salt reagent. From that time till November, 1939, the solutions were neutralized first before addition of the reagent (3), a procedure which was subsequently shown to give low results for some solutions (18).

From November, 1939, a reliable technique eliminating the use of caustic alkali has been used (19) and all the results here reported, unless otherwise indicated, have been obtained by this procedure.

Copper

The diethyldithiocarbamate reagent has been used throughout. Both the amyl alcohol extraction method (20) and Hoar's method of stabilizing the colour with gum arabic (21) have been used, the latter especially for amounts of copper in excess of 100 p.p.m.

Iron

Determinations of iron were made at the same time, using α, α^1-dipyridyl. As this reagent gives some colour with copper as well as with ferrous iron, appropriate corrections were made by using a separate tintometer graph. No great accuracy can be claimed for such a method (error about $\pm$ 10 per cent.) but tests gave good agreement with results by the thiocyanate method, also affected by copper, using as standards iron solutions containing comparable amounts of copper.

RESULTS

The results for both concentration and total amounts of cobalt, copper and iron in the livers, are summarized in Tables I to IV. Only results for livers from animals of known state of health are shown. Diagnostic material, from unthrifty animals affected by some unknown complaint not of parasitic origin, or samples submitted for cobalt status, are not included in the tables. Concentrations are reported as parts of the element per million (p.p.m.) of dry matter (constant weight at 105° c.), and total amounts are expressed in milligrams of the element. Where there are only two samples in the particular group, the cobalt, copper and iron figures are kept in correct relative order.

The effect of age of the animals on size of the liver makes it necessary for an examination of total cobalt levels to separate the animals into different age groups. For sheep, 3-9 months, 12-20 months, and 24 months and older were selected as these are the normal age groups at slaughter. For cattle the ages are less clearly defined, namely "yearlings" 6 months to 2 years, and "mature" beasts more than 2 years (usually more than 3 years).

DISCUSSION

COBALT IN LIVERS OF HEALTHY ANIMALS

As a basis for reference and discussion, values for normal healthy animals are considered first. Table V shows results for animals other than sheep and cattle.

(1) *Effect of age of animals on cobalt content of the liver.*

(a) *Sheep.*

In healthy sheep on a steady, natural, unsupplemented, cobalt diet some increase in concentration of cobalt from birth through to weaning is indicated from the results in Table I. The figures for two of the new-born lambs and the 2, 4 and 8 weeks lambs as well as 2 figures each for 3, 5 and 6 months lambs form a truly comparable series. These were from a uniform group of lambs born and raised on a paddock on the Horotiu-Te Kowhai formation at the Ruakura Animal Research Station, commencing from mid-August, 1939. These animals received no cobalt supplements of any kind, while the evidence from analysis of pastures

in the previous season is that the cobalt content of the pasture would be fairly constant (see reference (22) Table II, p. 97B), though slightly higher at the time the new-born and six months lambs were killed. No highly significant differences in cobalt concentration were found comparing the new-born, two weeks, and four weeks lambs with three months lambs, but compared with six months lambs the differences were statistically significant at the 1 per cent. level.

When the figures in Table I for animals 3 months and older are further analysed, we find in the 3-9 months age group 59 samples averaging 0.159 p.p.m. (range 0.063 to 0.322); 12-20 months 5 samples averaging 0.155 p.p.m. (range 0.111-0.190); 2 years and older 6 samples averaging 0.252 p.p.m. (range 0.183-0.315). Of the 59 samples from healthy 3-9 months lambs, only 5, all less than 7 months old, showed figures of less than 0.10 p.p.m. cobalt. Although the number of samples in the oldest age group is small, the average cobalt level is appreciably higher than in the 3-9 months age group.

In healthy 3-9 months lambs total cobalt ranged from 0.010 to 0.050 mg., average 0.023 mg., in 12-20 months sheep 0.019 to 0.025, average 0.023 mg.; and for sheep 2 years and older 0.027 to 0.073, average 0.051 mg.

(b) *Cattle.*

In new-born and bobby calves the cobalt concentration is significantly lower than in healthy yearling and mature cattle beasts (Table I).

In new-born and bobby calves, total cobalt varied from 0.004 to 0.035 mg., average 0.014, while in six healthy yearlings the range was 0.129 to 0.168 mg., average 0.146.

(2) *Effect of cobalt level in the diet on cobalt content of the livers of healthy animals.*

(a) *No cobalt supplements.*

In healthy sheep in " healthy " districts, the few examples where relevant data on cobalt content of the pasture are available, indicate some correlation between cobalt concentration in the liver and dietary cobalt, especially where differences in dietary levels are pronounced. For sheep kept healthy in bush-sick country by means of cobalt supplements, see Table VI and subsequent discussion. The lowest of twelve results for lambs from the Wairarapa district, namely 0.063 p.p.m. (0.013 mg. total cobalt) was for an animal from Mt. Bruce district where Maunsell and Lamont (23) found low levels of cobalt in some pastures (0.04 to 0.06 p.p.m.), while the highest value, 0.26 p.p.m. (0.024 mg. total cobalt), was for a lamb from an area associated with relatively high cobalt levels in the pastures (approx. 0.25 p.p.m.). On the other hand a comparison of two groups of 5-6 months lambs from Ruakura, one grazing on a pasture which provided on a yearly average approximately 0.13 p.p.m. cobalt (paddock 13A, reference (22)), the other 0.20 p.p.m. cobalt, showed no statistically significant differences in concentration, though seven of the eight values showed consistent correlation.

(b) *Cobalt supplements.*

In healthy districts, animals which have had access to proprietary licks containing small amounts of cobalt gave an average cobalt concentration of 0.22 p.p.m. as compared with 0.17 for those receiving no supplements. Where drenches containing very small amounts of cobalt

have been used, the average figure is 0.18. In neither case is the difference significant, probably because the amount of cobalt used in such proprietary licks and drenches in healthy areas was initially very small. *Note:* Cobalt licks, used in the main deficiency areas contain higher levels of cobalt, namely the recommended amount of 4 oz. cobalt sulphate per ton (24), and can be expected to give greater differences.

Josland's work (16) has shown that much larger doses of cobalt result in greatly increased cobalt levels in the liver. With injurious amounts of cobalt, figures in excess of 3 p.p.m. cobalt have been obtained.

COBALT IN LIVERS OF BUSH-SICK ANIMALS

(Results are summarized in Table III.)

(1) *Effect of age of animals on cobalt content of the liver.*

Sheep.

The livers from the two foetal and new-born lambs from bush-sick ewes show concentrations of cobalt comparable with those in 3 months and older sheep affected by bush-sickness. Data from other animals of less certain history and not reported in the table confirm this trend. This means a progressive increase in total cobalt content roughly proportional to the weights of the livers. Five lambs 5-9 months old gave an average figure of 0.039 p.p.m. (range 0.029-0.045), 15 sheep 12-20 months 0.024 p.p.m. (range 0.008-0.040) and 10 sheep 2 years and older 0.041 p.p.m. (range 0.033 to 0.054).

Cattle.

No information is available on the effect of age on the cobalt content of livers of bush-sick cattle beasts.

(2) *Effect of cobalt level in the diet on cobalt content of the liver.*

The improvement in health of sheep and cattle on North Island bush-sick pastures from the use of cobalt supplements in the form of licks, drenches or topdressing has been referred to in various publications (24) to (32). The results of the extensive series of topdressing experiments instituted at Mamaku in 1938, show good correlations between cobalt content of the pasture and cobalt content of the liver, and both with incidence of deficiency symptoms (Table VI).

Twenty-three of the thirty samples from bush-sick animals reported in Table III were from these and earlier Mamaku experiments and all without exception were associated with pastures whose average cobalt content for monthly samples taken over the preceding 12 months did not exceed 0.06 p.p.m. cobalt (range 0.04 to 0.07, average 0.055 to 0.061). This means that figures of 0.008 to 0.054 p.p.m. cobalt, average 0.031 p.p.m., in the liver have been associated with an average yearly cobalt content of the pasture of 0.06 p.p.m. On the cobalt topdressed pastures, however, breeding ewes and lambs have been maintained in excellent condition. In these the cobalt content of the livers is relatively high, in accord with the increased cobalt content of the pasture.

DIAGNOSTIC VALUE OF COBALT CONTENT OF LIVERS

(1) *Correlations.*

The results in Tables I to III and VI show a fair correlation between both cobalt concentration and total cobalt in the liver and incidence of bush-sickness. The results of analyses of livers from animals suffering from other known ailments (Table IV) and also the unreported results

of analysis of diagnostic samples from unthrifty animals from " healthy " districts, which have shown quite high cobalt levels, confirm that the correlation is with cobalt deficiency and not with unthriftiness.

(a) *Animals at an age when they are particularly susceptible to bush-sickness.*

Sheep.

The livers of typical North Island bush-sick sheep 5 months and older contained 0.03 p.p.m. cobalt on dry matter (range 0.01 to 0.05), while normal healthy untreated sheep 3 months and older from healthy districts averaged 0.17 p.p.m. cobalt (range 0.06 to 0.32, most values being above 0.10 p.p.m.). When the figures for sheep 2 years and older are examined, we find a still more pronounced difference in cobalt concentration (average for 10 bush-sick ewes 0.041 p.p.m., range 0.033 to 0.054) as against 0.252 p.p.m. (range 0.183 to 0.315) for healthy ewes. Sheep kept healthy in bush-sick country by means of adequate cobalt supplements have shown still higher average levels (0.36 p.p.m.) though the values are more variable (range 0.08 to 1.2).

Similar or slightly lower cobalt levels in the livers of sheep affected by cobalt deficiency ailment similar to bush-sickness were obtained by South Island workers at Glenhope, Nelson (4, 6, 10) and at Morton Mains, Southland (4, 7, 8).

Total cobalt in the livers of typical bush-sick sheep 5-9 months old ranged from 0.001 to 0.008 mg. (average 0.004) as compared with 0.010 to 0.050 (average 0.023) for healthy 3-9 months sheep. For 12-20 months the figures were respectively 0.002 to 0.007 (average 0.004) and 0.019 to 0.025 (average 0.023), and for 24 months and older 0.004 to 0.012 (average 0.006) and 0.027 to 0.073 (average 0.051).

Cattle.

For bush-sick yearling heifers and steers the values for concentration of cobalt were 0.03 p.p.m. (range 0.02 to 0.04) and for healthy yearling or mature cattle beasts 0.24 p.p.m. (range 0.12 to 0.40).

Total cobalt in the liver of the one bush-sick yearling heifer for which total weight of the liver is available gave 0.04 mg. Co., as compared with an average of 0.15 mg. (range 0.13 to 0.17) for six healthy yearlings from healthy districts.

(b) *Animals which normally do not show symptoms of bush-sickness.*

Sheep.

From the very limited data available, supplemented by data not here tabulated from animals of less certain history, it appears that the cobalt concentration in the livers of foetal and new-born lambs from bush-sick ewes is similar to that of sheep 3 months and older. On the other hand, foetal and new-born lambs from healthy ewes in healthy districts (Table I) show an average cobalt level of 0.08 p.p.m. (range 0.05 to 0.12). New-born lambs from ewes kept healthy in bush-sick country by means of cobalt supplements show an average cobalt level in the liver of 0.15 p.p.m. (range 0.11 to 0.20).

Cattle.

No data are available for comparison of levels in new-born calves from bush-sick cows with those from healthy mothers.

Other animals.

In New Zealand cobalt deficiency is known only in sheep and cattle. Analyses of two samples from a case of suspected deficiency in goats on a farm at Mohaka on East Coast hill country associated with cobalt deficiency in sheep gave inconclusive results (Table V). Cunningham in 1937 (33) and Underwood and Elvehjem (34) were unable to produce cobalt deficiency in rats, possibly because in both cases the purified diets still contained cobalt (34, 35).

Anomalous results.

Where cobalt drenches have been given to bush-sick animals *in extremis* in an unsuccessful attempt to save their lives, the cobalt level in the liver may increase to normal levels. Samples from such animals or from any cobalt treated animals are usually unsuitable for diagnostic purposes.

The only serious anomalies in the reference data were for two livers from sheep from Okaihau, North Auckland (Table I, A (2)). Total cobalt could not be determined. Two samples from Taranaki which gave rather low results of 0.050 and 0.056 p.p.m., the latter 0.008 mg. total cobalt, have also been included in this section. Although these four animals were described as healthy, they were all from farms with histories of ailment consistent with mild cobalt deficiency.

Askew, in experiments at Glenhope in the South Island (6), concluded that a cobalt content of 0.05 p.p.m. on dry basis in the livers of 2½ to 3 year old sheep and 0.010 mg. cobalt per organ, is compatible with perfect health. Later Askew and Watson (10) demonstrated that the cobalt level in the liver drops to quite low levels before the development of typical deficiency symptoms. Furthermore, cobalt contents as low as 0.04 p.p.m. had been reported for healthy animals at Morton Mains in Southland (4). The conclusion from the present work, however, is that such low levels of cobalt, though sometimes associated with healthy animals, are indicative of sub-optimal or dangerously low amounts of cobalt in the diet. In diagnostic work, therefore, such figures are treated as indicative of deficiency of cobalt.

APPLICATION OF RESULTS

(1) *Cobalt concentrations.*

From an early stage in this investigation, tentative diagnoses were made with the following results :

As the highest cobalt figure yet noted in this work for the liver of a healthy sheep which had received no cobalt supplements is 0.32 p.p.m. and for cattle, 0.40 p.p.m., figures above 1 p.p.m. cobalt have been regarded as indicating abnormally high cobalt in the diet through access to cobalt other than that present in the untreated pasture.

Sheep.

Fifty-three cases in animals 4 months or older, which showed a range of cobalt concentrations from 0.015 to 0.060 p.p.m. were tentatively diagnosed as cobalt deficient. Of these, 38, including that associated with the 0.060 p.p.m. figure in a 6 months lamb, have been sustained by subsequent good results with other sheep from the use of cobalt supplements. In the remaining cases, information has been insufficient to justify any conclusion. In 6 out of 8 cases, tentatively classed as "border-line" (animals 6 months or older, range of cobalt concentrations 0.063 to 0.091 p.p.m.) there is evidence of subsequent benefit to other sheep in the

flocks from the use of cobalt. In one instance, two apparently similar lambs from the one draft gave values of 0.047 and 0.087 p.p.m. cobalt respectively. A controlled drenching experiment proved clearly the benefit of cobalt on the farm concerned. This variability of results due to " sampling error " shows that isolated analyses can be expected to give only an indication of the overall picture.

More than 50 cases have been diagnosed as healthy for cobalt, where the concentration has been in excess of 0.10 p.p.m. The large majority of these were from unthrifty animals, most of which had received no cobalt supplements and which were affected by unknown complaints apparently not of parasitic origin. Many were from districts where there is no evidence of cobalt deficiency.

Cattle.

All 5 deficiency diagnoses (range 0.051 to 0.080 p.p.m. in animals 9 months and older) have been sustained by subsequent good results from the use of cobalt. Two of 3 " border-line " cases in yearlings, where the figures ranged from 0.094 to 0.105 p.p.m. have subsequently been confirmed by responses to cobalt. In 3 other cases, this time in unthrifty 3 to 4 months calves, figures of 0.079, 0.100 and 0.103 p.p.m. were obtained. In none of these cases has the suspected marginal deficiency been confirmed.

All other samples from unthrifty animals, 9 months or older, which showed figures above 0.13 p.p.m. have been classified as healthy for cobalt.

(2) *Total cobalt.*

Though the correlation between total cobalt and incidence of bush-sickness is good, there appears to be no advantage in determining total cobalt except possibly to assist in interpretation of results in some cases where the liver is of unusual size.

DIAGNOSTIC CRITERIA

On the basis of the reference data here presented, the following criteria are suggested :

Sheep.

Three months and older, below 0.06 p.p.m. cobalt deficiency, above 0.10 p.p.m. adequate cobalt.

Less than 3 months, below 0.04 p.p.m. suspected cobalt deficiency, above 0.08 p.p.m. adequate cobalt.

Cattle.

Nine months and older, below 0.05 p.p.m. cobalt deficiency, above 0.12 p.p.m. adequate cobalt.

Less than 9 months, data inadequate, but lower levels are indicated, especially in new-born calves.

Evidence from the response to cobalt supplements of other animals on farms where figures intermediate between these limits have been obtained in diagnostic work suggests that values below 0.08 p.p.m. in sheep 6 months and older and below 0.10 p.p.m. in cattle 9 months and older are indicative of sub-optimal amounts of cobalt.

COPPER

Figures for copper have been recorded in Tables I to V. The main purpose of the analyses for copper was to ensure firstly that bush-sickness in the North Island of New Zealand was not associated with copper deficiency as in South Australia, and secondly to ensure that there was no possibility of copper deficiency in diagnostic cases referred to this Laboratory. The copper levels in bush-sick animals (Table III) confirm Aston's early findings (36) that typical bush-sickness on non-peaty pumice lands in the North Island is *not* associated with copper deficiency. On the contrary, the average copper level in bush-sick sheep 3 months and older was 460 p.p.m. as compared with 320 p.p.m. for healthy untreated animals from healthy districts, and 406 p.p.m. for healthy cobalt treated from healthy districts. The higher level in bush-sick areas is confirmed from analyses of livers of healthy treated animals from these same areas, where the average copper level found for sheep 3 months and older was 556 p.p.m. The figure of 460 p.p.m. for bush-sick sheep would be increased if the calculations were made on fat-free basis as several of the samples were from animals with enlarged fatty livers. In the South Island, Dixon (37) at Morton Mains and Askew (6) at Glenhope have obtained comparable figures for copper in ailing sheep.

Several cases of enzootic icterus in animals from the Mamaku Farm have been associated with very high copper figures in the liver, in excess of 1,000 p.p.m. on dry basis. Analyses by Piper's modification (38) of Sylvester and Lampitt's method (39) showed copper levels in two pasture samples from Mamaku control paddock No. 9 of 12.4 and 14.0 p.p.m. This indicates that the intake of copper at Mamaku may be higher than in the cobalt deficiency areas of Western Australia (40) where the average copper content in typical cases was only 3.7 p.p.m. (9.9 for holdings unaffected by enzootic marasmus).

Among diagnostic samples handled by the writer only one case of suspected combined cobalt and copper deficiency was noted, from Waipapa in North Auckland. This and any cases of suspected simple copper-deficiency, were referred to Dr. I. J. Cunningham for his copper investigations.

As published work on copper contents of livers indicated divergent opinions as to what constituted normal levels in the livers of sheep and cattle, the samples obtained for the cobalt survey of healthy districts were analysed for copper content for reference purposes.

In sheep there appears to be no very significant change in copper concentration from birth (average 288 p.p.m.) through to maturity (average 323 p.p.m., Table I), though the levels at weaning may be somewhat lower. On the other hand, livers of new-born and bobby calves show levels of copper similar to sheep but the copper concentration drops appreciably from birth through to yearling stage, a conclusion which has been arrived at by many other workers (41) to (46). The total copper content in consequence remains relatively constant. This investigation has revealed some low figures for copper in the livers of apparently healthy cattle beasts from country in which cobalt deficiency does not occur.

IRON

Enzootic marasmus in Western Australia (13), "coast disease" in South Australia (12), and some cases of "salt-sick" in Florida (47), are associated with marked deposition or accumulation of iron in the liver,

kidney and spleen. In coast disease the liver shows particularly high deposition of iron, the average reported by Marston *et al.* (12) for 13 untreated weaners showing 16,100 p.p.m. and for one ewe 12,000 p.p.m. iron. Nine sheep affected by enzootic marasmus showed in Underwood's work (13) an average iron content of 2,380 p.p.m. In order to compare New Zealand results with these findings, iron was determined on most of the samples analysed for cobalt and copper.

The average for 29 North Island (N.Z.) bush-sick sheep (Table III) was 360 p.p.m. (range 80 to 853) as compared with 180 p.p.m. (range 89 to 457) for healthy untreated sheep aged 3 months and older from healthy districts. Animals 6 months and older kept healthy on bush-sick country with cobalt supplements showed intermediate values, average 215 p.p.m. (range 110 to 303). Results in Table I show that the concentration and total amount of iron in the liver normally drop from birth through to weaning.

The average iron content in the livers of 20 bobby calves from healthy cows was 1,290 p.p.m. (range 67 to 4,380), compared with 300 p.p.m. (range 137 to 644) for 21 healthy yearlings and mature cattle beasts.

The low figures for iron in the livers of North Island bush-sick sheep (average 360 p.p.m.) are similar to those found by Dixon (37) in Morton Mains disease in the South Island of New Zealand and are in marked contrast with the high figures reported for cobalt deficiency ailments in Australia.

In coast disease the copper content ranged from 5 to 10 p.p.m. (12), in enzootic marasmus 14 to 22 p.p.m. (40), and in bush-sickness 60 to 1,220 p.p.m. (Table III), suggesting an inverse relation between concentration of copper and extent of accumulation of iron in the livers. Underwood and Beck's results (40) indicate that the excessive stores of iron in the tissues of affected animals are used up during recovery from enzootic marasmus while being treated with cobalt only. It would, therefore, appear that the iron mobilizing effects of cobalt and copper are supplementary, with copper playing the dominant role. Thus iron is immobilized in the liver when there is either a severe deficiency of copper, or a moderate deficiency of copper and a deficiency of cobalt.

CONCLUSIONS

(a) Good correlation is evident between both cobalt concentration and total cobalt content in the liver and incidence of bush-sickness in sheep and cattle. In general the correlation is considered to be better than was obtained from analysis of pastures. There appears to be no additional advantage from determining total cobalt as well as concentration.

(b) Sheep affected by facial eczema, copper deficiency, enzootic icterus, unthrifty sheep and cattle from healthy areas, and cattle affected by copper deficiency, show cobalt levels similar to healthy animals, confirming that the correlation is with cobalt deficiency and not with unthriftiness due to any cause.

(c) Four abnormally low figures for cobalt in livers of healthy sheep have been observed but all appear to be from marginal deficiency areas.

(d) The average figure for copper in livers of typical bush-sick sheep is somewhat higher than normal for New Zealand and very much higher than typical examples of enzootic marasmus or coast disease.

In the normal sheep livers examined there appears to be no significant change in copper concentration from birth through to maturity. In calves the copper concentration drops appreciably from birth to yearling stage.

(e) The average iron content in the livers of bush-sick sheep is higher than that found in healthy normals but accumulation of iron is very slight compared with enzootic marasmus and coast disease. Similar trends are found in the limited data for cattle. The iron mobilizing effects of cobalt and copper appear to be supplementary, with copper playing the dominant role.

(f) Neither copper nor iron contents can be used as an indicator of cobalt deficiency in New Zealand.

(g) For diagnostic purposes the following criteria have been successfully employed.

(i) For sheep 3 months and older, concentrations of cobalt below 0.06 p.p.m. indicate cobalt deficiency, above 0.10 p.p.m. sufficiency of cobalt.

(ii) For yearling and mature cattle beasts figures below 0.05 p.p.m. indicate cobalt deficiency and above 0.12 p.p.m. adequate cobalt.

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TABLE 1. HEALTHY SHEEP AND CATTLE WHICH HAVE RECEIVED NO COBALT SUPPLEMENTS

(A) SHEEP.	Number of Samples.			Concentration (p.p.m.). Cobalt.		Copper.		Iron.		Number of Samples.			Total Metal (mg.). Cobalt.		Copper.		Iron.	
(I) *Healthy Districts.*	Co	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.	Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.
1. Foetal lambs from healthy ewes (a) 3 months	4	4	4	.051-.107	.074	67-132	111	107-3920	2330	2	2	2	.0006 .0010	.0008	1.0,1.2	1.1	32,26	29
(b) full term.	4			.073-.091	.083													
2. New-born lambs from healthy ewes.	4	4	4	.060-.117	.083*	163-440	288	880-8400	3630	4	4	4	.0008-.0016	.0014	2.9-6.0	4.6	20-114	53
3. Fourteen days lambs, as last.	2	2	2	.088,.096	.092	110,150	130	1720,3190	2460	2	2	2	.0029,.0029	.0029	3.7,4.6	4.2	76,98	87
4. Four and eight weeks lambs, as last	4	4	4	.107-.141	.120	64-285	189	104-1750	554	4	4	4	.0052-.0149	.0095	6.7-24.8	14	8.5-86	32
5. Sheep 3 months and older	70	70	16	.063-.322	.167	101-1370	323	89-457	182	67	67	43	.010-.073	.026	15-117	47	11-64	26
(II) *Apparently Healthy†, untreated, from borderline areas.*																		
1. North Auckland (1 year).	2	2	2	.029,.036	.033	500,150	325	119,252	186									
2. Taranaki (4 months).	2	2	2	.050,.056	.053	288,382	335	108,194	151	1	1	1	-,.008		-,52		-,26	
(B) CATTLE.																		
1. 7 months to full term foetus from healthy cows, no cobalt supplements.	7	7	7	.097-.162	.127	406-724	503	2080-6840	3630	7	7	7	.0050-.0122	.0078	24-49	31	121-513	228
2. Healthy new-born calves, as last.	20			.031-.147	.075					20			.004-.030	.012				
3. "Bobby" calves (2 to 5 days) as above	18	17	14	.074-.226	.119	139-502	337	67-4360	1270	14	13	11	.007-.035	.016	21-80	46	10-689	181
4. Cattle beasts, 6 months and older.	33	33	21	.122-.398	.240	6‡-191	49	137-644	302	6	6		.129-.168	.146	6-116	63		

* Figure of 0.50 reported in early work (16) suspected too high due to contamination from high cobalt samples analysed about the same time.
† Apparently healthy though from farms with histories of ailment consistent with cobalt deficiency.
‡ Though these animals were described as healthy, Cunningham's investigations (14) raise a query about the copper status of several.

TABLE II.

HEALTHY SHEEP AND CATTLE WHICH HAVE RECEIVED COBALT SUPPLEMENTS

	Concentration (p.p.m.).									Total Metal (mg.).								
	Number of Samples.			Cobalt.		Copper.		Iron.		Number of Samples.			Cobalt.		Copper.		Iron.	
(A) SHEEP.																		
(I) *Healthy Districts.*	Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.	Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.
(a) *Access to Cobalt Licks* (Small amounts of Cobalt).																		
Sheep 3 months and older.	19	16	8	.114-.348	.216	70-768	282	84-466	182	18	15	7	.018-.071	.034	12-145	42	10-85	28
(b) *Cobalt drenched* (Small amounts of Cobalt included in drench).																		
Sheep 3 months and older.	7	6	5	.128-.280	.182	166-1630	736	122-1740	719	5	4	3	.016-.041	.027	24-188	86	18-53	33
(c) *Combining* (a) *and* (b).	26	22	13	.114-.348	.207	70-1630	406	84-1740	388	23	19	10	016-.071	.032	12-188	51	10-85	30
(II) *Cobalt Deficiency Areas.*																		
(a) *Cobalt topdressed pastures.*																		
1. New-born lambs from healthy ewes	8	8	8	.105-.200	.151	40-320	167	250-1070	518	8	8	8	.0018-.0052	.0033	0.8-6.5	3.6	4-35	13
2. Healthy 3 weeks lamb as (1).	1	1	1	.112		149		83		1	1	1	.0038		5.0		3	
3. 4 years old ewes.*	9	8	8	.078-1.17	.485	169-706	476	110-303	225	9	8	8	.019-.204	.095	43-153	98	20-77	49
(b) *Access to licks containing small amounts of Cobalt* (wethers).	2	2	2	.168,.220	.194	432,720	576	348,130	239	2	2	2	.025,.022	.023	65,71	68	52,13	32
(c) *Cobalt drenched.*																		
1. New-born lambs from healthy ewes drenched. 1 mg. Co. twice weekly. (Pdk. 9).	2	2	2	.085,.126	.106	212,280	246	235,1420	828	2	2	2	.0011,.0031	.0021	2.8,6.9	4.8	3.1,35	19
2. Healthy 18 days lamb as above.	1	1	1	.116		143		238		1	1	1	.0048		6.0		9.9	
3. 6 months lambs drenched several weeks as in (1).	4	4	1	.097-.305	.162	445-738	603	182		4	4	1	.012-.035	.019	52-85	70	21	
4. 4 years ewe, as last, drenched 10 months	1	1	1	.408		896		262		1	1	1	.106		233		68	
5. 18 months wethers, 140 mg. Co. once monthly, 9 months.	2	2	2	.417,.497	.457	473,830	652	203,152	178	2	2	2	.134,.117	.125	151,196	174	65,36	51
6. Combining (a) 3, (b) and (c) 3-5.	18	17	14	.078-1.17	.357	169-896	556	110-303	215	18	17	14	.012-.204	.074	43-233	105	13-77	46
(B) CATTLE.																		
Healthy Districts.																		
Access to Cobalt Licks.																		
1. "Bobby" calves from cows with access to licks.	7	7	6	.075-.141	.107	159-532	352	263-4380	1400	4	4	3	.008-.016	.013	32-60	46	30-70	45
2. 4 years ox.	1	1	1	.184		79		149										

* Most of these ewes died from lambing trouble but none showed evidence of cobalt deficiency.

TABLE III.

BUSH-SICK SHEEP AND CATTLE (UNTREATED)

	Number of Samples.			Concentration (p.p.m.).						Number of Samples			Total Metal (mg.).							
				Cobalt.		Copper.		Iron.					Cobalt		Copper.		Iron.			
	Co	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.	Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.		
(1) SHEEP.																				
(a) Foetal lamb from bush-sick ewe.	1	1	1	.021		450		2720		1	1	1	.00007		3.8		23			
(b) New-born lamb from bush-sick ewe.	1			.034						1			.00029							
(c) Sheep 3 months and older	30	29	29	.008-.054	.032	60-1220	460	80-853	363	26	25	25	.0012-.0122	.0046	5-159	66	18-159	56		
(2) CATTLE.																				
Bush-sick yearlings.	3	3	3	.025-.038	.034	122-92	109	746,420	718	1	1	1	.037 (.038		90 (92		41 (420			
				.038		113		987					p.p.m.)		p.p.m.)		p.p.m.)			

TABLE IV. SHEEP AND CATTLE AFFECTED BY AILMENTS OTHER THAN BUSH-SICKNESS

	Number of Samples.			Concentration (p.p.m.).						Number of Samples.			Total Metal (mg.).					
	Cobalt.			Copper.		Iron.				Cobalt.			Copper.		Iron.			
SHEEP.	Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.	Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.
(1) *Facial Eczema.*																		
Sheep 3 months and older.	6	6	6	.073-.132	.091*	272-685	532	92-271	176	3	3	3	.010-.016	.013	35-121	71	15-43	26
(2) *Copper Deficiency.*																		
(a) New-born lambs from ewes in copper deficient areas.	5			.047-.132	.099					5			.0011-.0016	.0014				
(b) Ewes.	4			.166-.282	.205					4			.025-.059	.042				
(3) *Enzootic Icterus.*																		
(a) Country normally healthy for cobalt (6 months and older).	10	14	13	.057-.243	.122*	1080-3520	2160	90-1610	1020		4	4			113-207	144	80-144	107
(b) Bush-sick country.																		
1. Apparently cobalt deficient also.	1	1	1	.018*		1650		536										
2. Cobalt supplements.	4	4	4	.051-.098	.077*	1450-2140	1820	620-1390	1020	1	1	1	.0075		165		54	
(4) *Fed Excess Copper.*†																		
1g. $CuSO_4.5H_2O$ daily till death.	5	6	6	.040-.110	.068*	2090-4320	3030	55-1370	468									
CATTLE.																		
Copper Deficiency.																		
20 months and older (no Cobalt supplements).	8			.134-.307	.228					8			.115-.573	.379				

* Analyses by earlier less reliable method (3), liable to be low.
† Cases of experimentally induced jaundice—M. Buddle's experiments at Wallaceville (48).

TABLE V

OTHER ANIMALS

	Number of Samples.			Concentration (p.p.m.).						Number of Samples.			Total Metal (mg.).					
				Cobalt.		Copper.		Iron.					Cobalt.		Copper.		Iron.	
A. *Goats.*	Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.	Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.
(1) Healthy wild kid.	1	1	1	.222		110		160		1			.0057		2.8		4.1	
(2) Suspected cobalt deficiency.																		
(a) Kid goat.	1	1	1	.203		17		112					.014		1.2		8	
(b) Nanny goat (4 years).	1	1	1	.170		18		184					.016		1.7		17	
B. *Pigs.*																		
Healthy 4-9 months.	8	8	8	.068-.190	.102	13-37	23	428-804	641	8	8	8	.007-.040	.014	1.2-5.2	3.0	40-196	88
C. *Horse.*																		
Healthy.	1			.196														
D. *Rabbit.*	1			.34		16		720					.0032		0.15		6.7	
E. *Rats.*																		
Healthy untreated.	18	12	22	.14-.24	.18*	12-18	15	240-1280	725				.00016-.00047	.0003	.012-.055	.039	0.30-1.9	1.2

* See (49). Figure of 1.4 p.p.m. reported in earlier work for Josland (15) suspected of contamination from high cobalt samples

Table VI. Correlation of Cobalt Content of Mamaku and Waimiha Pastures and Cobalt Content of Livers with Bush-sickness

Description.	Number of Samples.	Cobalt content (p.p.m.) on dry matter.			
		Pasture.		Liver.	
		Range.	Yearly Av.	Range.	Average.
Mamaku.					
Bush-sick* lamb	4	0.04-0.07	0.06	0.039-0.045	0.042
,, hoggets	12	0.04-0.07	0.06	0.008-0.040	0.024
,, ewes	3	0.04-0.07	0.06	0.034-0.054	0.044
Healthy 2-tooth	1	0.06-0.11	0.08		0.103
Healthy 4 yrs. ewes†	1	0.05-0.62	0.16 (approx.)		0.38
,, ,,	3	0.05-1.2	0.18 ,,	0.078-1.17	0.61
,, ,,	2	0.08-0.62	0.27 ,,	0.25-0.29	0.27
,, ,,	1	0.08-2.8	0.63 ,,		1.05
Waimiha.					
Bush-sick ewes	6	0.04-0.08	0.06 ,,	0.033-0.045	0.040
Healthy ewes	6	0.10-1.03	0.50 ,,	0.35-0.80	0.52

* About half of these bush-sick animals died, the balance being killed in a weak or unthrifty condition and showing typical symptoms of severe deficiency.

† These ewes died from lambing trouble but were otherwise in good condition. Their weight and general health records ruled out the possibility of bush-sickness.

BACILLUS MESENTERICUS: AN ASSAY ORGANISM FOR PENICILLIN

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(*Received for publication, 13th August, 1947*)

Summary

The use is described of *Bacillus mesentericus* as a test organism in the plate method of penicillin assay. Its advantages over *Staphylococcus* are indicated.

Introduction

In the determination of the potency of penicillin solutions by the Heatley plate method it is usual to employ a suitably sensitive strain of *Staphylococcus*. This organism is, however, subject to certain disadvantages; of these the more important have been noted by Foster and Woodruff (1943 a), (1944). Most of the drawbacks mentioned by these authors have been experienced in this laboratory and more especially has it been found that staphylococci show a physiological variability from time to time. In addition the growth is usually not sufficiently opaque to give a good zone contrast and many strains tend to flocculate markedly in broth thus finally producing irregular growth in test plates. This has been observed more particularly with the culture of *S. aureus* strain "H" in our possession.

Foster and Woodruff (1943 b) in introducing the use of *Bacillus subtilis* as a test organism claim that it obviates most of the failings of staphylococcus.

Two years ago when it became evident at Wallaceville that *S. aureus* was not entirely suitable for penicillin assays it was decided to test a number of organisms of the *B. subtilis* group. Of these our strains of *B. subtilis, B. mycoides* and *B. megatherium* proved insufficiently sensitive and failed in other respects to fulfil the requirements of the test. A fourth type, namely, *B. mesentericus,* gave such successful results that it has since been employed here in routine penicillin determinations.

THE STRAIN

In 1942, during the course of a bacteriological examination of some pig feed, *Bacillus mesentericus* was isolated together with numbers of other typical soil flora. This was the source of the strain now used at Wallaceville for assay work. Culturally and biochemically it duplicates the characters assigned to *Bacillus mesentericus vulgatus* by Topley and Wilson (1936) except that nitrates in broth are not reduced. Bergey (1939) states, however, that nitrate reduction may or may not occur within this species.

THE ASSAY METHOD

The assay procedure adopted here differs in no essential respect from those usually employed. Ordinary 2.5 per cent. nutrient agar is used as a medium; this is distributed in 100 ml. lots in flasks. An 18 hour nutrient broth culture of *B. mesentericus* is used as an inoculum at the rate of 0.5 ml. of culture per 100 ml. of agar, and is added to the pre-melted agar at 46° C. This gives a density of organisms such that after incubation the colonies are almost but not quite confluent. A quantity of 12 ml. of the sown agar is apportioned to each plate, after which, without preliminary drying, the test solutions are added. The plates are incubated at 37° C. for 14 to 18 hours.

Incubation produces a highly opaque bacterial growth; the zone margins are clearly defined and the marked contrast between areas of growth and of non-growth facilitates accurate measurement of the zone diameters. The sensitivity of *B. mesentericus* between 0.5 and 1.5 units per ml. is roughly equivalent to that of *S. aureus* strain "H," but while the sensitivity curve follows the usual shape it tends to be flatter than that of the latter test organism. Penicillin potencies as low as $\frac{1}{8}$ unit per ml. are easily determined using the cork-borer (No. 3) method of agar-excision; this level of concentration gives a zone diameter of about 12.2 mm., while the diameter at a concentration of 5 units per ml., which is about the upper practicable limit, is approximately 26 mm.

The advantages of *B. mesentericus* may be summarized as follows: (1) physiological and cultural stability in time and in different batches of media; (2) excellent definition of inhibitory zones; (3) absence of the ring formation which frequently results from bacterial lysis in the case of *S. aureus;* (4) homogenous growth in nutrient broth without flocculation or pellicle formation (no surface growth appears until 4 to 6 days); (5) it is non-pathogenic; (6) owing to rapid growth, zones are measurable after from 8 to 10 hours of incubation.

On blood agar *B. mesentericus* is strongly Beta-haemolytic and this property suggests it would be a suitable organism for use in the tube assay method of Rammelkamp (1942).

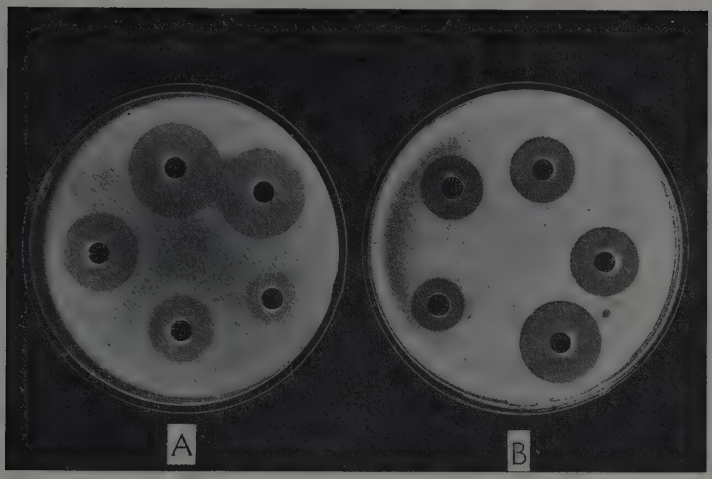

FIG. 1

The illustration (Fig. 1) shows an assay using (A) *S. aureus* strain "H" and (B) *Bacillus mesentericus* in which the zone clarity can be seen down to the lowest concentration of penicillin. It should be stated that the particular plate of *S. aureus* was selected as being the best of several dozens whereas that of *B. mesentericus* is quite typical.

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CONTROL OF HALO-BLIGHT OF BEANS

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Summary

In 1945-46 and 1946-47 seasons dwarf beans infected with halo-blight (*Pseudomonas medicaginis* var. *phaseolicola* Stapp and Kotte) were treated with Bordeaux (6-8-100) and Cuprox (copper oxychloride, 5-100).

Treatments consisted of two, three and four applications of each spray material.

Because of low rainfall, incidence of halo-blight in 1945-46 season was low but all treatments reduced infection to a very small amount. In 1946-47 season results showed that three and four applications reduced disease to a negligible amount but that two applications were not as effective.

Bordeaux and Cuprox were of approximately equal value for control of halo-blight.

Green-pod plus seed yields of treated plots were approximately double those of untreated plots.

INTRODUCTION

IN a previous paper (Reid and Taylor, 1945) results of copper spray treatments for control of halo-blight (*Pseudomonas medicaginis* var. *phaseolicola* Stapp and Kotte*) were given. These showed that four applications each of Bordeaux and of Cuprox (copper oxychloride 50 per cent. copper) reduced infection to a negligible amount and that two and three applications, though not as effective, appreciably limited infection. To check these findings, results of which are reported in present paper, further trials were carried out in 1945-46 and 1946-47 seasons.

METHODS

In each season an area of ground 140 feet by 60 feet was sown with Masterpiece dwarf bean seed in 29 drills 24 inches apart. After germination plants were thinned to 3 in. and the area was divided into 49 plots each of four rows 16 feet in length. Treatments were arranged in the form of a 7 x 7 latin square. Every fourth row served temporarily as an infection row, and was removed as soon as halo-blight was established. When plants had formed the first true leaves, the three permanent rows of each plot, with the exception of check plots, received a first application of spray material. After the spray had dried, the whole area, including infection rows and check plots, was sprayed with a water suspension of halo-blight bacteria. In the 1945-46 season infection rows were removed on 14th January and in 1946-47 season on 30th December.

Treatments consisted of two, three and four applications of (1) Cuprox (5 lb. per 100 gallons water) and (2) Bordeaux (6 lb. copper sulphate, 8 lb. hydrated lime, 100 gallons water) applied with a knapsack pump.

Times of application were arranged to give maximum protection from seedling stage to time of picking green pods, a total period estimated as approximately 36 days. This period was divided according to the number of applications intended; thus there were intervals of 18, 12 and 9 days between applications for the two-, three- and four-spray treatments respectively. In the 1945-46 season the first application of all treatments was given on 4th January and the last, that of the four-spray treatment, on 4th February. In the 1946-47 season the first application was given on 11th December and the last on 8th January.

The amount of spray required for a good cover increased with age of plants, the first application taking approximately ¾ gallon for seven plots of a treatment and the last 3 gallons for the same area of 74.6 square yards.

Periodical counts of plant and pod infection were recorded. Infected plants were graded 0 to 5 (Reid, 1945) relative to amount of disease present, 0 representing freedom from disease and 5 severe infection and possibly stunting of plant. Ten plants distributed through

* In previous paper designated *Pseudomonas medicaginis* (Sackett) Dowson. According to the most recent classification, this species is now *P. phaseolicola* (Burkholder) Dowson.

each row were so graded and the "infection index" in Table I gives average plant infection in each treatment. Pods of marketable size were picked on two occasions and were weighed, counted and recorded as infected or free from disease. In the second season further growth of pods occurred after the second picking and these were later harvested and the seed weighed.

RESULTS

1945-46 Season

Early examination of plants in infected rows showed an even distribution of halo-blight over the area. In this season there was no further increase in incidence after the initial infection. On 23rd January, 19 days after inoculation, check plots showed an average "infection index" of 3.2 and treated plots varied from 0.4 to 0.5 for Bordeaux and 0.6 to 0.9 for Cuprox treatments. The greater the number of spray applications the smaller was the index figure. The amount of disease on harvested pods indicated a similar gradation; checks showed 14.2 per cent. infected on 7th February and 8.9 per cent. at picking on 18th February; treated plots averaged 0.2 per cent. and 0.05 per cent. infection at these two picking dates respectively. Differences between results of both plant and pod infection of check and treatment plots were highly significant but no significant differences were apparent between treatments. Green-pod yields varied from 186 ounces per plot for checks and three- and four-spray Cuprox treatments to 216 ounces for the two-spray Bordeaux treatment. This difference in yield of 30 ounces was just significant at the 5 per cent. level.

1946-47 Season

Results of the 1946-47 season are given in Table I. In the three- or four-spray plots, infection regularly consisted of a single small lesion on each pod, whereas in check plots many lesions or extensive infected areas occurred on majority of pods. Pods from check plots were useless for marketing.

TABLE I. EFFECTS OF 2, 3 AND 4 APPLICATIONS OF COPPER SPRAY MATERIALS ON CONTROL OF HALO-BLIGHT OF BEANS

Treatment.	Number of Applications.	Halo-blight Infection; Treatment Averages.			Yields; Plot Averages.	
		Plant Infection Index 13/1/47.	Pods; Per cent. Infection 21/1/47.	27/1/47.	Pods; Oz. per Plot 21 & 27/1/47.	Seed; Oz. per Plot 26/2/47.
Cuprox 5-100	2	0.3	1.48	21.58	408.6	37.3
	3	0.2	0.23	10.91	390.1	33.1
	4	0.1	0.22	6.43	365.9	34.7
Bordeaux 6-8-100	2	0.9	2.23	27.94	412.4	39.8
	3	0.1	0.45	10.59	376.0	38.4
	4	0.1	0.26	8.77	394.8	37.8
Check. No Spray	–	4.1	75.58	56.88	204.9	4.1
Standard Error		0.1	0.93	2.30	25.1	4.3
Diff. for Significance at 5 per cent. Level		0.3	2.67	6.65	72.5	12.5

DISCUSSION

Although the amount ot disease present in the 1945-46 trials was small, as shown by the check plots, treatments markedly reduced plant and pod infection. The low incidence of halo-blight in a susceptible variety is unusual and in 1945-46 was apparently associated with low rainfall. In the three months covered by the 1945-46 trials the rainfall was 2.18, 0.82, and 0.51 inches, while in the following season for the three months of the trial it was 5.33, 2.91 and 2.67 inches. The average pod infection in check plots in the two periods were 11.5 per cent. and 66.2 per cent. respectively.

The results for 1946-47 also show that spray applications effectively checked bacterial infection of plants and pods. At the same time heavy infection in check plots decreased pod yield to approximately half that of treated plots. Seed yield was similarly affected though in this case the difference was greater. Bordeaux and Cuprox were of nearly equal value in preventing infection of plants and pods. The number of applications, however, brought about marked differences in amounts of infection. The two-spray figures of both Bordeaux and Cuprox on 27th January and of Bordeaux on 13th January, were significantly greater than those of other treatments on these dates. There were no significant differences between results of three- and four-spray treatments. The two-spray treatments show higher pod yields than do the three- or four-spray treatments. The differences might have arisen from variation in plant damage associated with number of spray applications. However, they are not significant at the 5 per cent. level, and from a practical point of view, the advantages of the higher yields are offset by the greater amount of disease present.

Whilst the above results are highly satisfactory it is probable that under commercial conditions, which would not include infection rows or untreated check plots, spray treatment would give an even better control.

In Table I, records of pod infection on 27th January show much higher figures than those of 21st January. This was because the period between the last application of treatments and picking of pods was unexpectedly prolonged by slow maturing of crop. Thus adequate protection was not maintained during later stages of pod development. This could be rectified either by an additional late spray or by adjusting times of application to give more uniform protection over the whole growing period.

CONCLUSIONS

The results, together with those previously reported (Reid and Taylor, 1945) show that halo-blight of beans can be readily controlled by use of Bordeaux and Cuprox sprays. At least three applications are advisable and the intervals between them should be adjusted to give protection from the seedling stage to crop harvest.

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